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TITLE: Compositions and methods for treating infections using cationic peptides alone or in combination with antibiotics

Abstract Text (1):
Compositions and methods for treating infections, especially bacterial infections, are provided. Indolicidin peptide analogues containing at least two basic amino acids are prepared. The analogues are administered as modified peptides, preferably containing photo-oxidized solubilizer.

Brief Summary Text (2):
The present invention relates generally to methods of treating microorganism-caused infections using cationic peptides or a combination of cationic peptides and antibiotic agents, and more particularly to using these peptides and antibiotic agents to overcome acquired resistance, tolerance, and inherent resistance of an infective organism to the antibiotic agent.

Brief Summary Text (4):
For most healthy individuals, infections are irritating, but not generally life-threatening. Many infections are successfully combated by the immune system of the individual. Treatment is an adjunct and is generally readily available in developed countries. However, infectious diseases are a serious concern in developing countries and in immunocompromised individuals.

Brief Summary Text (5):
In developing countries, the lack of adequate sanitation and consequent poor hygiene provide an environment that fosters bacterial, parasitic, fungal and viral infections. Poor hygiene and nutritional deficiencies may diminish the effectiveness of natural barriers, such as skin and mucous membranes, to invasion by infectious agents or the ability of the immune system to clear the agents. As well, a constant onslaught of pathogens may stress the immune system defenses of antibody production and phagocytic cells (e.g., polymorphic neutrophils) to subnormal levels. A breakdown of host defenses can also occur due to conditions such as circulatory disturbances, mechanical obstruction, fatigue, smoking, excessive drinking, genetic defects, AIDS, bone marrow transplant, cancer, and diabetes. An increasingly prevalent problem in the world is opportunistic infections in individuals who are HIV positive.

Brief Summary Text (6):
Although vaccines may be available to protect against some of these organisms, vaccinations are not always feasible, due to factors such as inadequate delivery mechanisms and economic poverty, or effective, due to factors such as delivery too late in the infection, inability of the patient to mount an immune response to the vaccine, or evolution of the pathogen. For other pathogenic agents, no vaccines are available. When protection against infection is not possible, treatment of infection is generally pursued. The major weapon in the arsenal of treatments is antibiotics. While antibiotics have proved effective against many bacteria and thus saved countless lives, they are not a panacea. The overuse of antibiotics in certain situations has promoted the spread of resistant bacterial strains. And of great importance, antibacterials are useless against viral infections.

Brief Summary Text (8):
Although cationic peptides show efficacy in vitro against a variety of pathogenic cells including gram-positive bacteria, gram-negative bacteria, and fungi, these peptides are generally toxic to mammals when injected, and therapeutic indices are usually quite small. Approaches to reducing toxicity have included development of a derivative or delivery system that masks structural elements involved in the toxic response or that improves the efficacy at lower doses. Other approaches under evaluation include liposomes and micellular systems to improve the clinical effects of peptides, proteins, and hydrophobic drugs, and cyclodextrins to sequester hydrophobic surfaces during administration in aqueous media. For example, attachment of polyethylene glycol (PEG) polymers, most often by modification of amino groups, improves the medicinal value of some proteins such as asparaginase and adenosine deaminase, and increases circulatory half-lives of peptides such as interleukins.

Brief Summary Text (11):
In addition neither antibiotic therapy alone of cationic peptide therapy alone can effectively combat all infections. By expanding the categories of microorganisms that respond to therapy, or by overcoming the resistance of a microorganism to antibiotic agents, health and welfare will be improved. Additionally quality of life will be improved, due to, for example, decreased duration of therapy, reduced hospital stay including high-care facilities, with the concomitant reduced risk of serious nosocomial (hospital-acquired) infections.

Brief Summary Text (16):
In other aspects, the invention provides an isolated nucleic acid molecule whose sequence comprises one or more coding sequences of the indolicidin analogues, expression vectors, and host cells transfected or transformed with the expression vector.

Brief Summary Text (19):
In yet another aspect, the invention provides a method of treating an infection, comprising administering to a patient a therapeutically effective amount of a pharmaceutical composition. The infection may be caused by, for example, a microorganism, such as a bacterium (e.g., Gram-negative or Gram-positive bacterium or anaerobe; parasite or virus.

Brief Summary Text (24):
The invention also provides a pharmaceutical composition comprising at least one modified compound and a physiologically acceptable buffer, and in certain embodiments, further comprises an antibiotic agent, antiviral agent, an antiparasitic agent, and/or antifimgal agent. The composition may be used to treat an infection, such as those caused by a microorganism (e.g., bacterium, fungus, parasite and virus).

Brief Summary Text (25):
This invention also generally provides methods for treating infections caused by a microorganism using a combination of cationic peptides and antibiotic agents. In one aspect, the method comprises administering to a patient a therapeutically effective dose of a combination of an antibiotic agent and a cationic peptide, wherein administration of an antibiotic agent alone is ineffective. Preferred peptides are provided.

Brief Summary Text (26):
In another aspect, a method of enhancing the activity of an antibiotic agent against an infection in a patient caused by a microorganism is provided, comprising administering to the patient a therapeutically effective dose of the antibiotic agent and a cationic peptide. In yet another aspect, a method is provided for enhancing the antibiotic activity of lysozyme or nisin, comprising administering lysozyme or nisin with a cationic peptide.

Brief Summary Text (27):
In other aspects, methods of treating an infection in a patient caused by a bacteria that is tolerant to an antibiotic agent, caused by a microorganism that is inherently resistant to an antibiotic agent; or caused by a microorganism that has acquired resistance to an antibiotic agent; comprises administering to the patient a

therapeutically effective dose of the antibiotic agent and a cationic peptide, thereby overcoming tolerance, inherent or acquired resistance to the antibiotic agent.

Drawing Description Text (2):
FIG. 1 is an SDS-PAGE showing the extraction profile of inclusion bodies (ib) from whole cells containing MBI-11 fusion protein. The fusion protein band is indicated by the arrow head. Lane 1, protein standards; lane 2, total lysate of XL1 Blue without plasmid; lane 3, total lysate of XL1 Blue (pR2h-11, pGP1-2), cultivated at 30.degree. C.; lane 4, total lysate of XL1 Blue (pR2h-11, pGP1-2), induced at 42.degree. C.; lane 5, insoluble fraction of inclusion bodies after Triton X100 wash; lane 6, organic extract of MBI-11 fusion protein; lane 7, concentrated material not soluble in organic extraction solvent.

Drawing Description Text (9):
FIG. 8 presents graphs showing the activity of MBI 11B7CN against mid-log cells grown in terrific broth (TB) or Luria-Bretani broth (LB).

Drawing Description Text (18):
FIG. 17 is a graph showing the number of animals surviving an MSSA infection after intraperitoneal injection of MBI 10CN, ampicillin, or vehicle.

Drawing Description Text (19):
FIG. 18 is a graph showing the number of animals surviving an MSSA infection after intraperitoneal injection of MBI 11 CN, ampicillin, or vehicle.

Drawing Description Text (20):
FIG. 19 is a graph showing the results of in vivo testing of MBI-11A1CN against S. aureus (Smith). Formulated peptide at various concentrations is administered by ip injection one hour after infection with S. aureus (Smith) by ip injection.

Drawing Description Text (21):
FIG. 20 is a graph showing the results of in vivo testing of MBI-11E3CN against S. aureus (Smith). Formulated peptide at various concentrations is administered by ip injection one hour after infection with S. aureus (Smith) by ip injection.

Drawing Description Text (22):
FIG. 21 is a graph showing the results of in vivo testing of: MBI-11F3CN against S. aureus (Smith). Formulated peptide at various concentrations is administered by ip injection one hour after infection with S. aureus (Smith) by ip injection.

Drawing Description Text (23):
FIG. 22 is a graph showing the results of in vivo testing of MBI-11G2CN against S. aureus (Smith). Formulated peptide at various concentrations is administered by ip injection one hour after infection with S. aureus (Smith) by ip injection.

Drawing Description Text (24):
FIG. 23 is a graph showing the results of in vivo testing of MBI-11CN against S. aureus (Smith). Formulated peptide at various concentrations is administered by ip injection one hour after infection with S. aureus (Smith) by ip injection.

Drawing Description Text (25):
FIG. 24 is a graph showing the results of in vivo testing of MBI-11B1CN against S. aureus (Smith). Formulated peptide at various concentrations is administered by ip injection one hour after infection with S. aureus (Smith) by ip injection.

Drawing Description Text (26):
FIG. 25 is a graph showing the results of in vivo testing of MBI-11B7CN against S. aureus (Smith). Formulated peptide at various concentrations is administered by ip injection one hour after infection with S. aureus (Smith) by ip injection.

Drawing Description Text (27):
FIG. 26 is a graph showing the results of in vivo testing of MBI-11B8CN against S. aureus (Smith). Formulated peptide at various concentrations is administered by ip injection one hour after infection with S. aureus (Smith) by ip injection.

Drawing Description Text (28):
FIG. 27 is a graph showing the results of in vivo testing of MBI-11G4CN against S. aureus (Smith). Formulated peptide at various concentrations is administered by ip injection one hour after infection with S. aureus (Smith) by ip injection.

Drawing Description Text (29):
FIGS. 28A and 28B display a graph showing the number of animals surviving an S. epidermidis infection after intravenous injection of MBI 10CN, gentamicin, or vehicle. Panel A, i.v. injection 15 min post-infection; panel B, i.v. injection 60 min post-infection.

Drawing Description Text (30):
FIG. 29 is a graph showing the number of animals surviving an MRSA infection mice after intravenous injection of MBI 11 CN, gentamicin, or vehicle.

Detailed Description Text (7):
As used herein, a "peptide analogue", "analogue", or "variant" of a cationic peptide, such as indolicidin, is at least 5 amino acids in length, has at least one basic amino acid (e.g., arginine and lysine) and has anti-microbial activity. Unless otherwise indicated, a named amino acid refers to the L-form. Basic amino acids include arginine, lysine, histidine and derivatives. Hydrophobic residues include tryptophan, phenylalanine, isoleucine, leucine, valine, and derivatives.

Detailed Description Text (12):
As noted above, this invention provides methods of treating infections caused by a microorganism, methods of killing a microorganism, and methods of enhancing the activity of an antibiotic agent. In particular, these methods are especially applicable when a microorganism is resistant to an antibiotic agent, by a mechanism, such as tolerance, inherent resistance, or acquired resistance. In this invention, infections are treated by administering a therapeutically effective dose of a cationic peptide alone or in combination with an antibiotic agent to a patient with an infection. Similarly, the combination can be contacted with a microorganism to effect killing.

Detailed Description Text (38):
Peptides may alternatively be synthesized by recombinant production (see e.g., U.S. Pat. No. 5,593,866). A variety of host systems are suitable for production of the peptide analogues, including bacteria (e.g., E. coli), yeast (e.g., Saccharomyces cerevisiae), insect (e.g., Sf9), and mammalian cells (e.g., CHO, COS-7). Many expression vectors have been developed and are available for each of these hosts. Generally, bacteria cells and vectors that are functional in bacteria are used in this invention. However, at times, it may be preferable to have vectors that are functional in other hosts. Vectors and procedures for cloning and expression in E. coli are discussed herein and, for example, in Sambrook et al. (Molecular Cloning.: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1987) and in Ausubel et al. (Current Protocols in Molecular Biology, Greene Publishing Co., 1995).

Detailed Description Text (39):
A DNA sequence encoding a cationic peptide is introduced into an expression vector appropriate for the host. In preferred embodiments, the gene is cloned into a vector to create a fusion protein. The fusion partner is chosen to contain an anionic region, such that a bacterial host is protected from the toxic effect of the peptide. This protective region effectively neutralizes the antimicrobial effects of the peptide and also may prevent peptide degradation by host proteases. The fusion partner (carrier protein) of the invention may further function to transport the fusion peptide to inclusion bodies, the periplasm, the outer membrane, or the extracellular environment. Carrier proteins suitable in the context of this invention specifically include, but are not limited to, glutathione-S-transferase (GST), protein A from Staphylococcus aureus, two synthetic IgG-binding domains (ZZ) of protein A, outer membrane protein F, .beta.-galactosidase (lacZ), and various products of bacteriophage .lambda. and bacteriophage T7. From the teachings provided herein, it is apparent that other proteins may be used as carriers. Furthermore, the entire carrier protein need not be used, as long as the protective anionic region is

present. To facilitate isolation of the peptide sequence, amino acids susceptible to chemical cleavage (e.g., CNBr) or enzymatic cleavage (e.g., V8 protease, trypsin) are used to bridge the peptide and fusion partner. For expression in E. coli, the fusion partner is preferably a normal intracellular protein that directs expression toward inclusion body formation. In such a case, following cleavage to release the final product, there is no requirement for renaturation of the peptide. In the present invention, the DNA cassette, comprising fusion partner and peptide gene, may be inserted into an expression vector, which can be a plasmid, virus or other vehicle known in the art. Preferably, the expression vector is a plasmid that contains an inducible or constitutive promoter to facilitate the efficient transcription of the inserted DNA sequence in the host. Transformation of the host cell with the recombinant DNA may be carried out by Ca.sup.++ -mediated techniques, by electroporation, or other methods well known to those skilled in the art.

Detailed Description Text (42):
Within a preferred embodiment, the vector is capable of replication in bacterial cells. Thus, the vector may contain a bacterial origin of replication. Preferred bacterial origins of replication include f1-ori and col E1 ori, especially the ori derived from pUC plasmids. Low copy number vectors (e.g., pPD100) may also be used, especially when the product is deleterious to the host.

Detailed Description Text (43):
The plasmids also preferably include at least one selectable marker that is functional in the host. A selectable marker gene confers a phenotype on the host that allows transformed cells to be identified and/or selectively grown. Suitable selectable marker genes for bacterial hosts include the chloroamphenicol resistance gene (Cm.sup.r), ampicillin resistance gene (Amp.sup.r), tetracycline resistance gene (Tc.sup.r) kanamycin resistance gene (Kan.sup.r), and others known in the art. To function in selection, some markers may require a complementary deficiency in the host.

Detailed Description Text (45):
The vector may also contain a gene coding for a repressor protein, which is capable of repressing the transcription of a promoter that contains a repressor binding site. Altering the physiological conditions of the cell can depress the promoter. For example, a molecule may be added that competitively binds the repressor, or the temperature of the growth media may be altered. Repressor proteins include, but are not limited to the E. coli lacI repressor (responsive to induction by IPTG), the temperature sensitive .lambda.cI857 repressor, and the like.

Detailed Description Text (55):
Monoclonal antibodies may also be readily generated from hybridoma cell lines using conventional techniques (see U.S. Pat. Nos. RE 32,011, 4,902,614, 4,543,439, and 4,411,993; see also Antibodies. A Laboratory Manual, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988). Briefly, within one embodiment, a subject animal such as a rat or mouse is injected with peptide, generally administered as an emulsion in an adjuvant such as Freund's complete or incomplete adjuvant in order to increase the immune response. The animal is generally boosted at least once prior to harvest of spleen and/or lymph nodes and immortalization of those cells. Various immortalization techniques, such as mediated by Epstein-Barr virus or fusion to produce a hybridoma, may be used. In a preferred embodiment, immortalization occurs by fusion with a suitable myeloma cell line to create a hybridoma that secretes monoclonal antibody. Suitable myeloma lines include, for example, NS-1 (ATCC No. TIB 18), and P3X63--Ag 8.653 (ATCC No. CRL 1580). The preferred fusion partners do not express endogenous antibody genes. After about seven days, the hybridomas may be screened for the presence of antibodies that are reactive against a telomerase protein. A wide variety of assays may be utilized (see Antibodies: A Laboratory Manual, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988).

Detailed Description Text (60):
Generally, cationic peptides are initially tested for (1) anti-microbial activity in vitro; (2) in vitro toxicity to normal mammalian cells; and (3) in vivo toxicity in an animal model. Peptides that have some anti-microbial activity are preferred, although such activity may not be necessary for enhancing the activity of an antibiotic agent. Also, for in vivo use, peptides should preferably demonstrate

acceptable toxicity profiles, as measured by standard procedures. Lower toxicity is preferred. Additional assays may be performed to demonstrate that the peptide is not immunogenic and to examine antimicrobial activity in vivo.

Detailed Description Text (65):
Candidate cationic peptides may be further tested for their toxicity to normal mammalian cells. An exemplary assay is a red blood cell (RBC) (erythrocyte) hemolysis assay. Briefly, in this assay, red blood cells are isolated from whole blood, typically by centrifugation, and washed free of plasma components. A 5% (v/v) suspension of erythrocytes in isotonic saline is incubated with different concentrations of peptide analogue. Generally, the peptide will be in a suitable formulation buffer. After incubation for approximately 1 hour at 37.degree. C., the cells are centrifuged, and the absorbance of the supernatant at 540 nm is determined. A relative measure of lysis is determined by comparison to absorbance after complete lysis of erythrocytes using NH.sub.4 Cl or equivalent (establishing a 100% value). A peptide with <10% lysis at 100 .mu.g/ml is suitable. Preferably, there is <5% lysis at 100 .mu.g/ml. Such peptides that are not lytic, or are only moderately lytic, are desirable and suitable for further screening. Other in vitro toxicity assays, for example measurement of toxicity towards cultured mammalian cells, may be used to assess in vitro toxicity.

Detailed Description Text (70):
The antibiotic activity of selected peptides may be assessed in vivo for their ability to ameliorate microbial infections using animal models. A variety of methods and animal models are available. Within these assays, a peptide is useful as a therapeutic if inhibition of microorganismal growth compared to inhibition with vehicle alone is statistically significant. This measurement can be made directly from cultures isolated from body fluids or sites, or indirectly, by assessing survival rates of infected animals. For assessment of antibacterial activity several animal models are available, such as acute infection models including those in which (a) normal mice receive a lethal dose of microorganisms, (b) neutropenic mice receive a lethal dose of microorganisms or (c) rabbits receive an inoculum in the heart, and chronic infection models. The model selected will depend in part on the intended clinical indication of the analogue.

Detailed Description Text (71):
By way of example, in a normal mouse model, mice are inoculated ip or iv with a lethal dose of bacteria. Typically, the dose is such that 90-100% of animals die within 2 days. The choice of a microorganismal strain for this assay depends, in part, upon the intended application of the analogue, and in the accompanying examples, assays are carried out with three different Staphylococcus strains. Briefly, shortly before or after inoculation (generally within 60 minutes), analogue in a suitable formulation buffer is injected. Multiple injections of analogue may be administered. Animals are observed for up to 8 days post-infection and the survival of animals is recorded. Successful treatment either rescues animals from death or delays death to a statistically significant level, as compared with non-treatment control animals. Analogues that show better efficacy than indolicidin itself are preferred.

Detailed Description Text (85):
A synergistic combination of cationic peptide and antibiotic agent may permit a reduction in the dosage of one or both agents in order to achieve a similar therapeutic effect. This would allow smaller doses to be used, thus, decreasing the incidence of toxicity (e.g., from aminoglycosides) and lowering costs of expensive antibiotics (e.g., vancomycin). Concurrent or sequential administration of peptide and antibiotic agent is expected to provide more effective treatment of infections caused by micro-organisms (bacteria, viruses, fungi, and parasites). In particular, this could be achieved by using doses that the peptide or antibiotic agent alone would not achieve therapeutic success. Alternatively, the antibiotic agent and peptide can be administered at therapeutic doses for each, but wherein the combination of the two agents provides even more potent effects.

Detailed Description Text (96):
The combination of a cationic peptide with an antibiotic agent, for which a microorganism is inherently resistant (i.e., the antibiotic has never been shown to

be therapeutically effective against the organism in question), is used to overcome the resistance and confer susceptibility of the microorganism to the agent. Overcoming inherent resistance is especially useful for infections where the causative organism is becoming or has become resistant to most, if not all, of the currently prescribed antibiotics. Additionally, administering a combination therapy provides more options when toxicity of an antibiotic agent and/or price are a consideration.

Detailed Description Text (97):
Overcoming resistance can be conveniently measured in vitro. Resistance is overcome when the MIC for a particular antibiotic agent against a particular microorganism is decreased from the resistant range to the sensitive range (according to the National Committee for Clinical Laboratory Standards (NCCLS)) (see also, Moellering, in Principles and Practice of Infectious Diseases, 4th edition, Mandell et al., eds. Churchill Livingstone, NY, 1995). NCCLS standards are based on microbiological data in relation to pharmacokinetic data and clinical studies. Resistance is determined when the organism causing the infection is not inhibited by the normal achievable serum concentrations of the antibiotic agent based on recommended dosage. Susceptibility is determined when the organism responds to therapy with the antibiotic agent used at the recommended dosage for the type of infection and microorganism.

Detailed Description Text (103):
Lysozymes disrupt certain bacteria by cleaving the glycosidic bond between N-acetylglucosamine and N-acetylmuramic acid in the polysaccharide component of bacterial cell walls. However, lysozyme exhibits only weak antibacterial activity with a narrow spectrum of activity. The addition of cationic peptide may improve the effectiveness of this activity and broaden the spectrum of activity.

Detailed Description Text (106):
In vivo testing involves the use of animal models of infection. Typically, but not exclusively, mice are used. The test organism is chosen according to the intended combination of cationic peptide and antibiotic to be evaluated. Generally, the test organism is injected intraperitoneally (IP) or intravenously (IV) at 10 to 100 times the fifty percent lethal dose (LD.sub.50). The LD.sub.50 is calculated using a method described by Reed and Muench (Reed L J and Muench H. The American Journal of Hygiene, 27:493-7.). The antibiotic agent and the cationic peptide are injected IP, IV, or subcutaneously (SC) individually as well as in combination to different groups of mice. The antimicrobial agents may be given in one or multiple doses. Animals are observed for 5 to 7 days. Other models of infection may also be used according to the clinical indication for the combination of antibiotic agents.

Detailed Description Text (108):
Synergy between the cationic peptide and the antibiotic agent is assessed using a model of infection as described above. For a determination of synergy, one or more of the following should occur. The combination group should show greater survival rates compared to the groups treated with only one agent; the combination group and the antibiotic agent group have equivalent survival rates with the combination group receiving a lower concentration of antibiotic agent; the combination group has equivalent or better survival compared to an antibiotic agent group with a lower microorganismal load at various time points.

Detailed Description Text (152):
The biological properties of APO-modified therapeutics appear to be improved compared to unmodified therapeutics. For example, modified and unmodified peptides are compared. Because the product consists of a peptide of known composition coupled to one or more polyoxyalkylene components derived from a polymeric mixture, defining an exact molecular weight for concentration calculations is not readily achieved. It is possible, however, to determine the concentration by spectrophotometric assay. Such a measurement is used to normalize APO-peptide concentrations for biological assays. For example, a 1 mg/mL MBI11CN-Tw80 solution contains the same amount of cationic peptide as a 1 mg/mL solution of the parent peptide, thus allowing direct comparison of toxicity and efficacy data. The modified peptides have an equivalent MIC to unmodified peptides. In vivo, however, the modified peptides demonstrate a lower LC.sub.50 than the unmodified peptides against a panel of tumor cell lines.

Thus, formation of APO-peptides increases the potency of cationic peptides against cancer cells in culture.

Detailed Description Text (155):
As noted above, the present invention provides methods for treating and preventing infections by administering to a patient a therapeutically effective amount of a peptide analogue of indolicidin as described herein. Patients suitable for such treatment may be identified by well-established hallmarks of an infection, such as fever, pus, culture of organisms, and the like. Infections that may be treated with peptide analogues include those caused by or due to microorganisms. Examples of microorganisms include bacteria (e.g., Gram-positive, Gram-negative), fungi, (e.g., yeast and molds), parasites (e.g., protozoans, nematodes, cestodes and trematodes), viruses, and prions. Specific organisms in these classes are well known (see for example, Davis et al., Microbiology, 3.sup.rd edition, Harper & Row, 1980). Infections include, but are not limited to, toxic shock syndrome, diphtheria, cholera, typhus, meningitis, whooping cough, botulism, tetanus, pyogenic infections, dysentery, gastroenteritis, anthrax, Lyme disease, syphilis, rubella, septicemia and plague.

Detailed Description Text (156):
More specifically, clinical indications include, but are not limited to: 1/infections following insertion of intravascular devices or peritoneal dialysis catheters; 2/infection associated with medical devices or prostheses; 3/infection during hemodialysis; 4/S. aureus nasal and extra-nasal carriage; 5/burn wound infections; 6/surgical wounds, 7/acne, including severe acne vulgaris; 8/nosocomial pneumonia; 9/meningitis; 10/cystic fibrosis; 11/infective endocarditis; 12/osteomyelitis; and 13/sepsis in an immunocompromised host.

Detailed Description Text (157):
1/Infections following insertion of contaminated intravascular devices, such as central venous catheters, or peritoneal dialysis catheters. These catheters are cuffed or non-cuffed, although the infection rate is higher for non-cuffed catheters. Both local and systemic infection may result from contaminated intravascular devices, more than 25,000 patients develop device related bacteremia in the United States each year. The main organisms responsible are coagulase-negative staphylococci (CoNS), Staphylococcus aureus, Enterococcus spp, E. coli and Candida spp.

Detailed Description Text (159):
2/Infection associated with medical devices or prostheses, e.g. catheter, grafts, prosthetic heart valves, artificial joints, etc. One to five percent of indwelling prostheses become infected which usually requires removal or replacement of the prostheses. The main organisms responsible for these infections are CoNS and S. aureus.

Detailed Description Text (161):
3/Infection during hemodialysis. Infection is the second leading cause of death in patients on chronic hemodialysis. Approximately 23% of bacteremias are due to access site infections. The majority of graft infections are caused by coagulate-positive (S. aureus) and coagulate-negative staphylococci. To combat infection, the peptide alone or in combination with an antibiotic can be applied as an ointment or cream to the dialysis site prior to each hemodialysis procedure.

Detailed Description Text (162):
4/S. aureus nasal and extra-nasal carriage. Infection by this organism may result in impetigenous lesions or infected wounds. It is also associated with increased infection rates following cardiac surgery, hemodialysis, orthopedic surgery and neutropenia, both disease induced and iatrogenic. Nasal and extra-nasal carriage of staphylococci can result in hospital outbreaks of the same staphylococci strain that is colonizing a patient's or hospital worker's nasal passage or extra-nasal site. Much attention has been paid to the eradication of nasal colonization, but the results of treatment have been generally unsatisfactory. The use of topical antimicrobial substances, such as Bacitracin, Tetracycline, or Chlorhexidine, results in the suppression of nasal colonization, as opposed to its eradication.

Detailed Description Text (164):
5/Burn wound infections. Although the occurrence of invasive burn wound infections has been significantly reduced, infection remains the most common cause of morbidity and mortality in extensively burned patients. Infection is the predominant determinant of wound healing, incidence of complications, and outcome of burn patients. The main organisms responsible are Pseudomonas aeruginosa, S. aureus, Streptococcus pyogenes, and various gram-negative organisms. Frequent debridements and establishment of an epidermis, or a surrogate such as a graft or a skin substitute, is essential for prevention of infection.

Detailed Description Text (165):
The peptide alone or in combination with antibiotics can be applied to burn wounds as an ointment or cream and/or administered systemically. Topical application may prevent systemic infection following superficial colonization or eradicate a superficial infection. The peptide is preferably administered as a 0.5 to 2% cream or ointment. Application to the skin could be done once a day or as often as dressings are changed. The systemic administration could be by intravenous, intramuscular or subcutaneous injections or infusions. Other routes of administration could also be used.

Detailed Description Text (166):
6/Surgical wounds, especially those associated with foreign material, e.g. sutures. As many as 71% of all nosocomial infections occur in surgical patients, 40% of which are infections at the operative site. Despite efforts to prevent infection, it is estimated that between 500,000 and 920,000 surgical wound infections complicate the approximately 23 million surgical procedures performed annually in the United States. The infecting organisms are varied but staphylococci are important organisms in these infections.

Detailed Description Text (168):
7/Acne, including severe acne vulgaris. This condition is due to colonization and infection of hair follicles and sebaceous cysts by Propionibacterium acne. Most cases remain mild and do not lead to scarring although a subset of patients develop large inflammatory cysts and nodules, which may drain and result in significant scarring.

Detailed Description Text (170):
8/Nosocomial pneumonia. Nosocomial pneumonias account for nearly 20% of all nosocomial infections. Patients most at risk for developing nosocomial pneumonia are those in an intensive care units, patients with altered levels of consciousness, elderly patients, patients with chronic lung disease, ventilated patients, smokers and post-operative patients. In a severely compromised patient, multiantibiotic-resistant nosocomial pathogens are likely to be the cause of the pneumonia.

Detailed Description Text (175):
11/Infective endocarditis. Infective endocarditis results from infection of the heart valve cusps, although any part of the endocardium or any prosthetic material inserted into the heart may be involved. It is usually fatal if untreated. Most infections are nosocomial in origin, caused by pathogens increasingly resistant to available drugs. The main organisms responsible are Viridans streptococci, Enterococcus spp, S. aureus and CoNS.

Detailed Description Text (177):
12/Osteomyelitis. In early acute disease the vascular supply to the bone is compromised by infection extending into surrounding tissue. Within this necrotic and ischemic tissue, the bacteria may be difficult to eradicate even after an intense host response, surgery, and/or antibiotic therapy. The main organisms responsible are S. aureus , E. coli, and P. aeruginosa.

Detailed Description Text (179):
13/Sepsis in immunocompromised host. Treatment of infections in patients who are immunocompromised by virtue of chemotherapy-induced granulocytopenia and immunosuppression related to organ or bone marrow transplantation is always a big challenge. The neutropenic patient is especially susceptible to bacterial infection,

so antibiotic therapy should be initiated promptly to cover likely pathogens, if infection is suspected. Organisms likely to cause infections in granulocytopenic patients are: S. epidermidis, S. aureus , S. viridans, Enterococcus spp, E. coli, Klebsiella spp, P. aeruginosa and Candida spp.

Detailed Description Text (181):
Effective treatment of infection may be examined in several different ways. The patient may exhibit reduced fever, reduced number of organisms, lower level of inflammatory molecules (e.g., IFN-.gamma., IL-12, IL-1, TNF), and the like.

Detailed Description Text (182):
The in vivo therapeutic efficacy from administering a cationic peptide and antibiotic agent in combination is based on a successful clinical outcome and does not require 100% elimination of the organisms involved in the infection. Achieving a level of antimicrobial activity at the site of infection that allows the host to survive or eradicate the microorganism is sufficient. When host defenses are maximally effective, such as in an otherwise healthy individual, only a minimal antimicrobial effect may suffice. Thus, reducing the organism load by even one log (a factor of 10) may permit the defenses of the host to control the infection. In addition, clinical therapeutic success may depend more on augmenting an early bactericidal effect than on the long-term effect. These early events are a significant and critical part of therapeutic success, because they allow time for the host defense mechanisms to activate. This is especially true for life-threatening infections (e.g. meningitis) and other serious chronic infections (e.g. infective endocarditis).

Detailed Description Text (189):
Pharmaceutical compositions of the present invention are administered in a manner appropriate to the infection or disease to be treated. The amount and frequency of administration will be determined by factors such as the condition of the patient, the cause of the infection, and the severity of the infection. Appropriate dosages may be determined by clinical trials, but will generally range from about 0.1 to 50 mg/kg. The general range of dosages for the antibiotic agents are presented below.

Detailed Description Text (192):
The peptides, especially the labeled analogues, may be used in image analysis and diagnostic assays or for targeting sites in eukaryotic multicellular and single cell cellular organisms and in prokaryotes. As a targeting system, the analogues may be coupled with other peptides, proteins, nucleic acids, antibodies and the like.

Detailed Description Text (212):
Peptide analogues are alternatively produced by recombinant DNA technique in bacterial host cells. The peptide is produced as a fusion protein, chosen to assist in transporting the fusion peptide to inclusion bodies, periplasm, outer membrane or extracellular environment.

Detailed Description Text (216):
The plasmid pR2h-11, employing a T7 promoter, high copy origin of replication, Apr marker and containing the gene of the fusion protein, is co-electroporated with pGP1-2 into E. coli strain XL1-Blue. Plasmid pGP1-2 contains a T7 RNA polymerase gene under control of a lambda promoter and cl857 repressor gene. Fusion protein expression is induced by a temperature shift from 30.degree. C. to 42.degree. C. Inclusion bodies are washed with solution containing solubilizer and extracted with organic extraction solvent. Profiles of the samples are analyzed by SDS-PAGE. FIG. 1 shows the SDS-PAGE analysis and an extraction profile of inclusion body from whole cell. The major contaminant in the organic solvent extracted material is P-lactamase (FIG. 1). The expression level in these cells is presented in Table 10.

Detailed Description Text (217):
In addition, a low-copy-number vector, pPD100, which has a chloramphenicol resistance gene, is used to express MBI-11 in order to eliminate the need for using ampicillin, thereby reducing the appearance of .beta.-lactamase in extracted material. This plasmid allows selective gene expression and high-level protein overproduction in E. coli using the bacteriophage T7 RNA polymerase/T7 promoter system (Dersch et al., FEMS Microbiol. Lett. 123: 19-26, 1994). pPD100 contains a

chloramphenicol resistance gene (CAT) as a selective marker, a multiple cloning site, and an ori sequence derived from the low-copy-number vector pSC101. There are only about 4 to 6 copies of these plasmids per host cell. The resulting construct containing MBI-11 is called pPDR2h-11. FIG. 2 presents a gel electrophoresis analysis of the MBI-11 fusion protein expressed in this vector. Expression level of MBI-11 fusion protein is comparable with that obtained from plasmid pR2h-11. The CAT gene product is not apparent, presumably due to the low-copy-number nature of this plasmid, CAT protein is not expressed at high levels in pPDR2h-11.

Detailed Description Text (283):
Using this assay, peptide activity, measured by dye release, is shown to be extremely sensitive to the composition of the liposomes at many liposome to peptide ratios (L/P) (FIG. 7). Specifically, addition of cholesterol to liposomes composed of egg phosphotidylcholine (PC) virtually abolishes membrane permeabilizing activity of MBI 11CN, even at very high lipid to peptide molar ratios (compare with egg PC liposomes containing no cholesterol). This in vitro selectivity may mimic that observed in vitro for bacterial cells in the presence of mammalian cells.

Detailed Description Text (286):
An alternative method for measuring peptide-membrane interaction uses the E. coli strain ML-35 (Lehrer et al., J. Clin. Invest., 84. 553, 1989), which contains a chromosomal copy of the lacZ gene encoding .beta.-galactosidase and is permease deficient. This strain is used to measure the effect of peptide on the inner membrane through release of .beta.-galactosidase into the periplasm. Release of .beta.-galactosidase is measured by spectrophotometrically monitoring the hydrolysis of its substrate .alpha.-nitrophenol .beta.-D-galactopyranoside (ONPG). The maximum rate of hydrolysis (V.sub.max) is determined for aliquots of cells taken at various growth points.

Detailed Description Text (287):
A preliminary experiment to determine the concentration of peptide required for maximal activity against mid-log cells, diluted to 4.times.10.sup.7 C .FU/ml, yields a value of 50 .mu.g/ml, which is used in all subsequent experiments. Cells are grown in two different growth media, Terrific broth (TB) and Luria broth (LB) and equivalent amounts of cells are assayed during their growth cycles. The resulting activity profile of MBI 11B7CN is shown in FIG. 8. For cells grown in the enriched TB media, maximum activity occurs at early mid-log (140 min), whereas for cells grown in LB media, the maximum occurs at late mid-log (230 min). Additionally, only in LB, a dip in activity is observed at 140 min. This drop in activity may be related to a transition in metabolism, such as a requirement for utilization of a new energy source due to depletion of the original source, which does not occur in the more enriched TB media. A consequence of a metabolism switch would be changes in the membrane potential.

Detailed Description Text (288):
To test whether membrane potential has an effect on peptide activity, the effect of disrupting the electrochemical gradient using the potassium ionophore vancomycin is examined. Cells pre-incubated with vancomycin are treated with peptide and for MBI 10CN and MBI 11CN ONPG hydrolysis diminished by approximately 50% compared to no pre-incubation with vancomycin (FIG. 9). Another cationic peptide that is not sensitive to vancomycin is used as a positive control.

Detailed Description Text (292):
Cationic peptides are tested for toxicity towards eukaryotic cells by measuring the extent of lysis of mammalian red blood cells (RBC). Briefly, in this assay, red blood cells are separated from whole blood by centrifugation and washed free of plasma components. A 5% (v/v) washed red blood cell suspension is prepared in isotonic saline. An aliquot of peptide in formulation is then added and mixed in. After incubation at 37.degree. C. for 1 hour with constant agitation, the solution is centrifuged and the supernatant measured for absorbance at 540 nm to detect released hemoglobin. When compared with the absorbance for a 100% lysed standard, a relative measure of the amount of hemoglobin that has been released from inside the red blood cells is determined and hence the ability of the peptide/formulation to cause red blood cell lysis.

Detailed Description Text (295):
A combination of cationic peptide and antibiotic agent is tested for toxicity towards eukaryotic cells by measuring the extent of lysis of mammalian red blood cells. Briefly, red blood cells are separated from whole blood by centrifugation, washed free of plasma components, and resuspended to a 5% (v/v) suspension in isotonic saline. The peptide and antibiotic agent are pre-mixed in isotonic saline, or other acceptable solution, and an aliquot of this solution is added to the red blood cell suspension. Following incubation with constant agitation at 37.degree. C. for 1 hour, the solution is centrifuged, and the absorbance of the supernatant is measured at 540 nm, which detects released hemoglobin. Comparison to the A.sub.540 for a 100% lysed standard provides a relative measure of hemoglobin release from red blood cells, indicating the lytic ability of the cationic peptide and antibiotic agcnt combination.

Detailed Description Text (296):
A red blood cell (RBC) lysis assay is used to group peptides according to their ability to lyse RBC under standardized conditions compared with MBI 11CN and Gramicidin-S. Peptide samples and washed sheep RBC are prepared in isotonic saline with the final pH adjusted to between 6 and 7. Peptide samples and RBC suspension are mixed together to yield solutions that are 1% (v/v) RBC and 5, 50 or 500 .mu.g/ml peptide. The assay is performed as described above. Each set of assays also includes MBI 11CN (500 .mu.g/ml) and Gramicidin-S (5 .mu.g/ml) as "low lysis" and "high lysis" controls, respectively.

Detailed Description Text (304):
The in vitro lifetime of free peptides in plasma and in blood is determined by measuring the amount of peptide present after set incubation times. Blood is collected from sheep, treated with an anticoagulant (not heparin) and, for plasma preparation, centrifuged to remove cells. Formulated peptide is added to either the plasma fraction or to whole blood and incubated. Following incubation, peptide is identified and quantified directly by reversed phase HPLC or an antibody-based assay. The antibiotic agent is quantified by a suitable assay, selected on the basis of its structure. Chromatographic conditions are as described above. Extraction is not required as the free peptide peak does not overlie any peaks from blood or plasma.

Detailed Description Text (309):
Peptide levels in plasma in vivo are measured after iv or ip administration of 80-100% of the maximum tolerated dose of peptide analogue in either formulation C1 or D. MBI 11CN in formulation C1 is injected intravenously into the tail vein of CD11CRBR strain mice. At various times post-injection, mice are anesthetized and blood is drawn by cardiac puncture. Blood from individual mice is centrifuiged to separate plasma from cells. Plasma is then analyzed by reversed phase HPLC column. The resulting elution profiles are analyzed for free peptide content by UV absorbance at 280 nm, and these data are converted to concentrations in blood based upon a calibrated standard. Each data point represents the average blood level from two mice. In this assay, the detection limit is approximately 1 .mu./ml, less than 3% of the dose administered.

Detailed Description Text (334):
In contrast, mice given a lethal dose had completely normal tissues and organs, except for the liver and heart of the ip and iv dosed mice, respectively. In general, this damage is identified as disruption of the cells lining the blood vessels. It appears as though the rapid death of mice is due to this damage, and that the peptide did not penetrate beyond that point. Extensive damage to the hepatic portal veins in the liver and to the coronary arterioles in the heart was observed.

Detailed Description Text (338):
Cationic peptides are tested for their ability to rescue mice from lethal bacterial infections. The animal model used is an intraperitoneal (ip) inoculation of mice with 10.sup.6 -10.sup.8 Gram-positive organisms with subsequent administration of peptide. The three pathogens investigated, methicillin-sensitive S. aureus (MSSA), methicillin-resistant S. aureus (MRSA), or S. epidermidis are injected ip into mice. For untreated mice, death occurs within 12-18 hours with MSSA and S. epidermis and

within 6-10 hours with MRSA.

Detailed Description Text (339):
Peptide is administered by two routes, intraperitoneally, at one hour post-infection, or intravenously, with single or multiple doses given at various times pre- and post-infection.

Detailed Description Text (340):
MSSA infection. In a typical protocol, groups of 10 mice are infected intraperitoneally with a LD.sub.90-100 dose (5.2.times.10.sup.6 CFU/mouse) of MSSA (Smith, ATCC #19640) injected in brain-heart infusion containing 5% mucin. This strain of S. aureus is not resistant to any common antibiotics. At 60 minutes post-infection, MBI 10CN or MBI 11CN, in formulation D, is injected intraperitoneally at the stated dose levels. An injection of formulation alone serves as a negative control and administration of ampicillin serves as a positive control. The survival of the mice is monitored at 1, 2, 3 and 4 hrs post-infection and twice daily thereafter for a total of 8 days.

Detailed Description Text (341):
As shown in FIG. 17, MBI 10CN is maximally active against MSSA (70-80% survival) at doses of 14.5 to 38.0 mg/kg, although 100% survival is not achieved. Below 14.5 mg/kg, there is clear dose-dependent survival. At these lower dose levels, there appears to be an animal-dependent threshold, as the mice either die by day 2 or survive for the full eight day period. As seen in FIG. 18, MBI 11CN, on the other hand, rescued 100% of the mice from MSSA infection at a dose level of 35.7 mg/kg, and was therefore as effective as ampicillin. There was little or no activity at any of the lower dose levels, which indicates that a minimum bloodstream peptide level must be achieved during the time that bacteria are a danger to the host.

Detailed Description Text (344):
S. epidermidis infection. Peptide analogues generally have lower MIC values against S. epidermidis in vitro, therefore, lower blood peptide levels might be more effective against infection.

Detailed Description Text (345):
In a typical protocol, groups of 10 mice are injected intraperitoneally with an LD.sub.90-100 dose (2.0.times.10.sup.8 CFU/mouse) of S. epidermidis (ATCC #12228) in brain-heart infusion broth containing 5% mucin. This strain of S. epidermidis is 90% lethal after 5 days. At 15 mins and 60 mins post-infection, various doses of MBI 11CN in formulation D are injected intravenously via the tail vein. An injection of formulation only serves as the negitive control and injection of gentamicin serves as the positive control; both are injected at 60 minutes post-infection. The survival of the mice is monitored at 1, 2, 3, 4, 6 and 8 hrs post-infection and twice daily thereafter for a total of 8 days.

Detailed Description Text (346):
As shown in FIGS. 28A and 28B, MBI 11CN prolongs the survival of the mice. Efficacy is observed at all three dose levels with treatment 15 minutes post-infection, there is less activity at 30 minutes post-infection and no significant effect at 60 minutes post-infection. Time of administration appears to be important in this model system, with a single injection of 6.1 mg/kg 15 minutes post-infection giving the best survival rate.

Detailed Description Text (347):
MRSA infection. MRSA infection, while lethal in a short period of time, requires a much higher bacterial load than MSSA. In a typical protocol, groups of 10 mice are injecteded intraperitoneally with a LD.sub.90-100 dose (4.2.times.10.sup.7 CFU/mouse) of MRSA (ATCC #33591) in brain-heart infusion containing 5% mucin. The treatment protocols are as follows, with the treatment times relative to the time of infection:

Detailed Description Text (348):
MBI 11CN is injected intravenously in the tail vein in formulation D. Survival of mice is recorded at 1, 2, 3, 4, 6, 8, 10, 12, 20, 24 and 30 hrs post-infection and twice daily thereafter for a total of 8 days. There was no change in the number of

surviving mice after 24 hrs (FIG. 29).

Detailed Description Text (349):
The 1 mg/kg (20 min) treatment protocol, with injections 5 minutes apart centered on the infection time, delayed the death of the mice to a significant extent with one survivor remaining at the end of the study. The results presented in Table 39 suggest that a sufficiently high level of MBI 11CN maintained over a longer time period would increase the number of mice surviving. The 5 mg/kg and 1 mg/kg (2 hr) results, where there is no improvement in survivability over the negative control, indicates that injections 1 hour apart, even at a higher level, are not effective against MRSA.

Detailed Description Text (383):
In addition, APO-peptides and parent peptides are tested against a panel of cancer cell lines. Cell death is measured using the Cytotox (Promega) assay kit which measures the release of lactate dehydrogenase. As shown below the modified peptides had increased activity over the parent peptides.

Detailed Description Text (384):
PBL, peripheral blood lymphocytes; HUVEC, human umbilical vein endothelial cells; H460, non-small lung tumor; K562, chronic myelogenous leukemia; DoHH-2, B-cell cell lymphoma; P388, lymphocytic leukemia; P388ADR, lymphocytic leukemia, multidrug resistant; MCF-7, breast carcinoma; MCF-7ADR, breast carcinoma, multidrug resistant.

Detailed Description Paragraph Table (2):
(SEQ A29771 Zasloff, M. (1987) (Xenopus laevis) ID NO: 210) Magainin II Amphibian skin GIGKFLHSAKKFGKAFVGEIMNS* (SEQ A29771 Zasloff, M. (1987) (Xenopus laevis) ID NO: 211) PGLa Amphibian skin GMASKAGAIAGKIAKVALKAL* (SEQ ID X13388 Kuchler, K. et al., (1989) (Xenopus laevis) NO: 212) PGQ Amphibian stomach GVLSNVIGYLKKLGTGALNAVLKQ (SEQ Moore, K. S. et al., (1989) (Xenopus laevis) ID NO: 213) XPF Amphibian skin GWASKIGQTLGKIAKVGLKELIQPK P07198 Sures, I. And Crippa, M. (Xenopus laevis) (SEQ ID NO: 214) (1984) Mastoparans Mastoparan Wasp venom INLKALAALAKKIL* (SEQ ID NO: 215) P01514 Bernheimer, A. and Rudy, (Vespula lewisii) B. (1986) Melittins Melittin Bee venom GIGAVLKVLTTGLPALISWIKRKRQQ P01504 Tosteson, M. T. and (Apis mellifera) (SEQ ID NO: 216) Tosteson, D. C. (1984) Phormicins Phormicin A Nestling-suckling ATCDLLSGTGINHSACAAHCLLRGNRGG P10891 Lambert, J. et al., (1989) blowfly YCNGKGVCVCRN (SEQ ID NO: 217) (Phormia terranovae) Phormicin B Nestling-suckling ATCDLLSGTGINHSACAAHCLLRGNRGG P10891 Lambert, J. et al., (1989) blowfly YCNRKGVCVRN (SEQ ID NO: 218) (Phormia terranovae) Polyphemusins Polyphemusin I Atlantic horseshoe crab RRWCFRVCYRGFCYRKCR* (SEQ ID P14215 Miyata, T. et al., (1989) (Limulus polyphemus) NO: 219) Polyphemusin II Atlantic horseshoe crab RRWCFRVCYKGFCYRKCR* (SEQ ID P14216 Miyata, T. et al., (1989) (Limulus polyphemus) NO: 220) Protegrins Protegrin I Porcine leukocytes RGGRLCYCRRRFCVCVGR (SEQ ID S34585 Kokryakov, V. N. et al., (sus scrofa) NO: 221) (1993) Protegrin II Porcine leukocytes RGGRLCYCRRRFCICV (SEQ ID NO: 222) 534586 Kikryakov, V. N. et al., (sus scrofa) (1993) Protegrin III Porcine leukocytes RGGGLCYCRRRFCVCVGR (SEQ ID 534587 Kokryakov, V. N. et al., (sus scrofa) NO: 223) (1993) Royalisins Royalisin Royal Jelly VTCDLLSFKGQVNDSACAANCLSLGKAG P17722 Fujiwara, S. et al., (1990) (Apis mellifera) GHCEKGVCICRKTSFKDLWDKYF (SEQ ID NO: 224) Sarcotoxins Sarcotoxin IA Flesh fly GWLKKIGKKIERVGQHTRDATIQGLGIA P08375 Okada, M. and Natori S., (Sacrophaga peregrina) QQAANVAATAR* (SEQ ID NO: 225) (1985b) Sarcotoxin IB Flesh fly GWLKKIGKKIERVGQHTRDATIQVIGVA P08376 Okada, M. and Natori S., (Sacrophaga peregrina) QQAANVAATAR* (SEQ ID NO: 226) (1985b) Seminal Seminalplasmin Bovine seminal plasma SDEKASPDKHHRFSLSRYAKLANRLANP S08184 Reddy, E. S. P. and plasmins (Bos taurus) KLLETFLSKWIGDRGNRSV (SEQ ID Bhargava, P. M. (1979) NO: 227) Tachyplesins Tachyplesin I Horseshoe crab KWCFRVCYRGICYRRCR* (SEQ ID P23684 Nakamura, T. et al., (1988) (Tachypleus tridentatus) NO: 228) Tachyplesin II Horseshoe crab RWCFRVCYRGICYRKCR* (SEQ ID P14214 Muta, T. et al., (1990) (Tachypleus tridentatus) NO: 229) Thionins Thionin Barley leaf KSCCKDTLARNCYNTCRFAGGSRPVCAG S00825 Bohlmann, H. et al., BTH6 (Hordeum vulgare) ACRCKIISGPKCPSDYPK (SEQ ID (1988) NO: 230) Toxins Toxin 1 Waglers pit viper venom GGKPDLRPCIIPPCHYIPRPKPR (SEQ ID P24335 Schmidt, J. J. et al., (1992) (Trimeresurus wagleri) NO: 231) Toxin 2 Sahara scorpion VKDGYIVDDVNCTYFCGRNAYCNEECT P01484 Bontems, F., et al., (1991) (Androctonus australis KLKGESGYCQWASPYGNACYCKLPDHV Hector) RTKGPGRCH (SEQ ID NO: 232) Arigiolas and Pisano,

(1984). JBC 259, 10106; Argiolas and Pisano, (1985). JBC 260, 1437; Banerjee and Hansen, (1988). JBC 263, 9508; Bellamy et al., (1992). J. Appl. Bacter. 73, 472; Bernheimer and Rudy, (1986). BBA 864, 123; Bohlmann et al., (1988). EMBO J. 7, 1559; Bontems et al., (1991). Science 254, 1521; Bulet et al., (1991). JBC 266, 24520; Bulet et al. (1992). Eur. J. Biochem. 209, 977; Bulet et al., (1993). JBC 268, # 14893; Casteels et al., (1989). EMBO J. 8, 2387; Casteels et al., (1990). Eur J. Biochem. 187, 381; Cociancich et al., (1993). BBRC 194, 17; Creighton and Charles, (1987). J. Mol. Biol. 194, 11; Csordas and Michl, (1970). Monatsh Chemistry 101, 182; Diamond et al., (1991). PNAS 88, 3952; Dickinson et al., (1988). JBC 263, 19424; Eisenhauer et al., (1989). Infect. and Imm. 57, 2021; Frank et al., (1990). JBC 265, 18871; # Fujiwara et al., (1990). JBC 265, 11333; Galvez et al., (1989). Antimicrobial Agents and Chemotherapy 33, 437; Ganz et al., (1989). J. Immunol. 143, 1358; Gibson et al., (1991). JBC 266, 23103; Gudmundsson et al., (1991). JBC 266, 11510; Hanzawa et al., (1990). FEBS Letters 269, 413; Hastings et al., (1991). J. of Bacteriology 173, 7491; Hultmark et al., (1982). Eur. J. Biochem. 127, 207; Hurst, A. (1981). Adv. Appl. Micro. 27, # 85; Kaletta et al., (1989). Archives of Microbiology 152, 16; Kokryakov et al., (1993). FEBS Letters 327, 231; Kuchler et al., (1989) Eur. J. Biochem. 179, 281; Lambert et al., (1989). PNAS 86, 262, Lee et al., (1989). PNAS 86, 9159; Lehrer et al., (1991). Cell 64, 229; Miyata et al., (1989). J. of Biochem. 106, 663; Moore et al., (1991). JBC 266, 19851; Mor et al., (1991). Biochemistry 30, 8824; Muta et al., (1990). J. Biochem. 108, # 261; Nakamura et al., (1988). JBC 263, 16709; Nakamura et al., (1983). Infection and Immunity 39, 609; Okada and Natori (1985). Biochem. J. 229, 453; Reddy and Bhargava, (1979). Nature 279, 725; Reichhart et al., (1989). Eur. J. Biochem. 182, 423; Romeo et al., (1988). JBC 263, 9573; Samakovlis et al., (1991). EMBO J. 10, 163; Schmidt et al., (1992). Toxicon 30, 1027; Schweitz et al., (1989). Biochem. 28, 9708; Selsted et # al., (1983). JBC 258, 14485; Selsted et al., (1992). JBC 267, 4292; Simmaco et al., (1993). FEBS Letters 324, 159; Sures and Crippa (1984). PNAS 81, 380; Takada et al., (1984). Infect. and Immun. 44, 370; Tosteson and Tosteson, (1984). Biophysical J. 45, 112; Tryselius et al., (1992). Eur. J. Biochem. 204, 395; Xanthopoulos et al., (1988). Eur. J. Biochem. 172, 371; Yamashita and Saito, (1989). Infect. and Imm. 57, 2405; Zasloff, M. (1987). PNAS 84, 5449.

Detailed Description Paragraph Table (3):

TABLE 2 Class of Antibiotic Antibiotic Mode of Action PENICILLINS Natural Penicillin G, Blocks the formation Benzylpenicillin of new cell walls in Penicillin V, bacteria Phenoxymethylpenicillin Penicillinase resistant Methicillin, Nafcillin, Oxacillin Cloxacillin, Dicloxacillin Acylamino-penicillins Ampicillin, Amoxicillin Carboxy-penicillins Ticarcillin, Carbenicillin Ureido-penicillins Mezlocillin, Azlocillin, Piperacillin CARBAPENEMS Imipenem, Meropenem Blocks the formation of new cell walls in bacteria MONOBACTAMS Aztreonam Blocks the formation of new cell walls in bacteria CEPHALOSPORINS 1st Generation Cephalothin, Cefazolin Prevents formation of 2nd Generation Cefaclor, Cefamandole new cell walls in Cefuroxime, Cefonicid, bacteria Cefmetazole, Cefotetan; Cefprozil 3rd Generation Cefetamet, Cefoperazone Cefotaxime, Ceftizoxime Ceftriaxone, Ceftazidime Cefixime, Cefpodoxime, Cefsulodin 4th Generation Cefepime CARBACEPHEMS Loracarbef Prevents formation of new cell walls in bacteria CEPHAMYCINS Cefoxitin Prevents formation of new cell walls in bacteria QUINOLONES Fleroxacin, Nalidixic Inhibits bacterial DNA Acid Norfloxacin, synthesis Ciprofloxacin, Ofloxacin, Enoxacin Lomefloxacin, Cinoxacin TETRACYCLINES Doxycycline, Mino- Inhibits bacterial cycline, Tetracycline protein synthesis, binds to 30S ribosome subunit. AMINO- Amikacin, Gentamicin, Inhibits bacterial GLYCOSIDIES Kanamycin, Netilmicin, protein synthesis, Tobramycin, Strepto- binds to 30S mycin ribosome subunit MACROLIDES Azithromycin, Clarithro- Inhibits bacterial mycin, Erythromycin protein synthesis, binds to 50S ribosome subunit Derivatives of Erythromycin estolate, Erythromycin Erythromycin stearate Erythromycin ethyl-succinate Erythromycin gluceptate Erythromycin lacto- bionate GLYCOPEPTIDES Vancomycin, Teico- Inhibits cell wall planin synthesis, prevents peptidoglycan elongation. MISCELLANEOUS Chloramphenicol Inhibits bacterial protein synthesis, binds to 50S ribosome subunit. Clindamycin Inhibits bacterial protein synthesis, binds to 50S ribosome subunit. Trimethoprim Inhibits the enzyme dihydrofolate reductase, which activates folic acid. Sulfamethoxazole Acts as antimetabolite of PABA & inhibits synthesis of folic acid Nitrofurantoin Action unknown, but is concentrated in urine where it can act on urinary tract bacteria Rifampin Inhibits bacterial RNA polymerase Mupirocin Inhibits bacterial protein synthesis

Detailed Description Paragraph Table (13):
TABLE 10 % protein Fusion Mol. mass in whole % in inclusion % which is protein (kDa) cell lysate body extract MBI-11 peptide MBI-11 20.1 15 42 7.2

Detailed Description Paragraph Table (53):
TABLE 39 Time of Observation Percentage of Animals Surviving (Hours post-infection) No Treatment Treatment 6 50% 70% 8 0 40% 10 0 30% 12 0 20%

Detailed Description Paragraph Table (59):
TABLE 45 CELL LINE, LC.sub.50, .mu.g/mL .+-. S.E. MCF- Peptide PBL HUVEC H460 K562 DoHH-2 P388 P388ADR MCF-7 7ADR 11CN 57 >190 200 -- -- 30 25 11.8 .+-. 9 17 .+-. 1 11CN-Tw80 6 .+-. 6 16 .+-. 4 16 .+-. 4 -- -- 1.9 .+-. 5 3.5 .+-. 2 11 -- 11A3CN >500 >500 >500 >500 >500 >300 >300 -- -- 11A3CN-Tw80 12.7 .+-. 15 17 .+-. 9 15 .+-. 4 6 3.3 .+-. 0.05 5.6 .+-. 2 6.6 .+-. 3 28 13 11B7CN 24 .+-. 10 90 .+-. 23 26 .+-. 25 34 .+-. 25 16.5 .+-. 3 13.8 -- >700 -- 11B7CN-Tw80 3.8 .+-. 1 12.8 .+-. 8 >100 4.7 .+-. 3 3.3 .+-. 1 5.1 -- 12 -- 11E3CN 22 .+-. 11 117 .+-. 7 18 9 3.6 13.9 .+-. 3 7.9 .+-. 3 5.6 .+-. 2 5.3 .+-. 1 11E3CN-Tw80 4.5 .+-. 2 12.8 .+-. 2 8.2 .+-. 4 4.9 .+-. 3 3.5 .+-. 0.7 5.9 .+-. 3 8.4 .+-. 1 8.1 .+-. 5 7.6 .+-. 2 21A11 30 .+-. 15 184 .+-. 100 48 56 .+-. 33 9.8 .+-. 0.3 -- -- -- -- 21A11-Tw80 4.5 .+-. 4 17 .+-. 9.9 21 4.3 .+-. 2 4.7 .+-. 0.6 8.1 .+-. 3.4 9 18 -- 29 12 .+-. 10 10 12.6 .+-. 10 1 2.1 .+-. 0.5 1.4 .+-. 0.5 2 .+-. 0.2 4 .+-. 2 3.2 .+-. 1 29-Tw80 8.7 .+-. 6 9.3 .+-. 2 1.7 2.1 .+-. 0.5 4 .+-. 0.5 7.6 .+-. 2.4 7.6 .+-. 2 15.5 .+-. 6 9.1 .+-. 5

CLAIMS:

36. A method of treating a microbial infection, comprising administering to a patient a therapeutically effective amount of a pharmaceutical composition according to any one of claims 12 or 13.

37. A method of treating a microbial infection, comprising administering to a patient a therapeutically effective amount of a pharmaceutical composition according to claim 14.

38. A method of treating a microbial infection, comprising administering to a patient a therapeutically effective amount of a pharmaceutical composition according to claim 30.

39. A method of treating a microbial infection, comprising administering to a patient a therapeutically effective amount of a pharmaceutical composition according to claim 31.

40. A method of treating a microbial infection, comprising administering to a patient a therapeutically effective amount of a pharmaceutical composition according to claim 32.

41. A method of treating a microbial infection, comprising administering to a patient a therapeutically effective amount of a pharmaceutical composition according to claim 33.

42. The method of claim 36, wherein the infection is due to a microorganism.

43. The method of claim 37, wherein the infection is due to a microorganism.

44. The method of claim 39, wherein the infection is due to a microorganism.

45. The method of claim 41, wherein the infection is due to a microorganism.

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L7: Entry 9 of 20 File: USPT Mar 25, 2003

DOCUMENT-IDENTIFIER: US 6538106 B1
TITLE: Compositions and methods for treating infections using analogues of indolicidin

Abstract Text (1):
Compositions and methods for treating infections, especially bacterial infections, are provided. Indolicidin peptide analogues containing at least two basic amino acids are prepared. The analogues are administered as modified peptides, preferably containing photo-oxidized solubilizer.

Brief Summary Text (2):
The present invention relates generally to treatment of microorganism-caused infections, and more specifically, to compositions comprising indolicidin analogues, polymer-modified analogues, and their uses in treating infections.

Brief Summary Text (4):
For most healthy individuals, infections are irritating, but not generally life-threatening. Many infections are successfully combated by the immune system of the individual. Treatment is an adjunct and is generally readily available in developed countries. However, infectious diseases are a serious concern in developing countries and in immunocompromised individuals.

Brief Summary Text (5):
In developing countries, the lack of adequate sanitation and consequent poor hygiene provide an environment that fosters bacterial, parasitic, fungal and viral infections. Poor hygiene and nutritional deficiencies may diminish the effectiveness of natural barriers, such as skin and mucous membranes, to invasion by infectious agents or the ability of the immune system to clear the agents. As well, a constant onslaught of pathogens may stress the immune system defenses of antibody production and phagocytic cells (e.g., polymorphic neutrophils) to subnormal levels. A breakdown of host defenses can also occur due to conditions such as circulatory disturbances, mechanical obstruction, fatigue, smoking, excessive drinking, genetic defects, AIDS, bone marrow transplant, cancer, and diabetes. An increasingly prevalent problem in the world is opportunistic infections in individuals who are HIV positive.

Brief Summary Text (6):
Although vaccines may be available to protect against some of these organisms, vaccinations are not always feasible, due to factors such as inadequate delivery mechanisms and economic poverty, or effective, due to factors such as delivery too late in the infection, inability of the patient to mount an immune response to the vaccine, or evolution of the pathogen. For other pathogenic agents, no vaccines are available. When protection against infection is not possible, treatment of infection is generally pursued. The major weapon in the arsenal of treatments is antibiotics. While antibiotics have proved effective against many bacteria and thus saved countless lives, they are not a panacea. The overuse of antibiotics in certain situations has promoted the spread of resistant bacterial strains. And of great importance, antibacterials are useless against viral infections.

Brief Summary Text (8):
Although cationic peptides show efficacy in vitro against a variety of pathogenic cells including gram-positive bacteria, gram-negative bacteria, and fungi, these

peptides are generally toxic to mammals when injected, and therapeutic indices are usually quite small. Approaches to reducing toxicity have included development of a derivative or delivery system that masks structural elements involved in the toxic response or that improves the efficacy at lower doses. Other approaches under evaluation include liposomes and micellular systems to improve the clinical effects of peptides, proteins, and hydrophobic drugs, and cyclodextrins to sequester hydrophobic surfaces during administration in aqueous media. For example, attachment of polyethylene glycol (PEG) polymers, most often by modification of amino groups, improves the medicinal value of some proteins such as asparaginase and adenosine deaminase, and increases circulatory half-lives of peptides such as interleukins.

Brief Summary Text (13):
In other aspects, the invention provides an isolated nucleic acid molecule whose sequence comprises one or more coding sequences of the indolicidin analogues, expression vectors, and host cells transfected or transformed with the expression vector.

Brief Summary Text (15):
In yet another aspect, the invention provides a method of treating an infection, comprising administering to a patient a therapeutically effective amount of a pharmaceutical composition. The infection may be caused by, for example, a microorganism, such as a bacterium (e.g., Gram-negative or Gram-positive bacterium or anaerobe; examples are Acinetobacter spp., Enterobacter spp., E. coli, H. influenzae, K. pneumoniae, P. aeruginosa, S. marcescens and S. maltophilia, Bordetella pertussis; Brucella spp., Campylobacter spp., Haemophilus ducreyi; Helicobacter pylori; Legionella spp.; Moraxella catarrhalis; Neisseria spp.,. Salmonella spp.; Shigella spp. and Yersinia spp.; E. faecalis, S. aureus, E. faecium, S. pyogenes, S. pneumoniae and coagulase-negative staphylococci; Bacillus spp.; Corynebacterium spp., Diphtheroids: Listeria spp. and Viridans Streptococci.; Clostridium spp., Bacteroides spp. and Peptostreptococcus spp.; Borrelia spp.; Chlamydia spp., Mycobacterium spp., Mycoplasma spp.; Propionibacterium acne; Rickettsia spp.; Treponema spp. and Ureaplasma spp.) fungus (e.g., yeast and/or mold), parasite (e.g., protozoan, nematode, cestode and trematode, such as Babesia spp., Balantidium coli; Blastocystis hominis; Cryptosporidium parvum; Encephalitozoon spp., Entamoeba spp.; Giardia lamblia; Leishmania spp.; Plasmodium spp.; Toxoplasma gondii; Trichomonas spp. Trypanosoma spp, Ascaris lumbricoides; Clonorchis sinensis; Echinococcus spp.; Fasciola hepatica; Fasciolopsis buski; Heterophyes heterophyes; Hymenolepis spp.; Schistosoma spp.; Taenia spp. and Trichinella spiralis) or virus (e.g., Alphavirus; Arenavirus; Bunyavirus; Coronavirus; Enterovirus; Filovirus; Flavivirus; Hantavirus; HTLV-BLV; Influenzavirus; Lentivirus; Lyssavirus; Paramyxovirus; Reovirus; Rhinovirus and Rotavirus, Adenovirus; Cytomegalovirus; Hepadnavirus; Molluscipoxvirus; Orthopoxvirus; Papillomavirus; Parvovirus; Polyomavirus; Simplexvirus and Varicellovirus).

Brief Summary Text (20):
The invention also provides a pharmaceutical composition comprising at least one modified compound and a physiologically acceptable buffer, and in certain embodiments, further comprises an antibiotic agent, antiviral agent, an antiparasitic agent, and/or antifungal agent. The composition may be used to treat an infection, such as those caused by a microorganism (e.g.. bacterium, fungus, parasite and virus).

Drawing Description Text (2):
FIG. 1 is an SDS-PAGE showing the extraction profile of inclusion bodies (ib) from whole cells containing MBI-11 fusion protein. The fusion protein band is indicated by the arrow head. Lane 1, protein standards; lane 2, total lysate of XL1 Blue without plasmid; lane 3, total lysate of XL1 Blue (pR2h-1, pGP 1-2), cultivated at 30.degree. C.; lane 4, total lysate of XL1 Blue (pR2h-11, pGP1-2), induced at 42.degree. C.; lane 5, insoluble fraction of inclusion bodies after Triton X100 wash; lane 6, organic extract of MBI-11 fusion protein; lane 7, concentrated material not soluble in organic extraction solvent.

Drawing Description Text (9):
FIG. 8 presents graphs showing the activity of MBI 11B7CN against mid-log cells

grown in terrific broth (TB) or Luria-Bretani broth (LB).

Drawing Description Text (15):
FIG. 14 is a graph showing the number of animals surviving an MSSA infection after intraperitoneal injection of MBI 10CN, ampicillin, or vehicle.

Drawing Description Text (16):
FIG. 15 is a graph showing the number of animals surviving an MSSA infection after intraperitoneal injection of MBI 11CN, ampicillin, or vehicle.

Drawing Description Text (17):
FIG. 16 is a graph showing the results of in vivo testing of MBI-11A1CN against S. aureus (Smith). Formulated peptide at various concentrations is administered by ip injection one hour after infection with S. aureus (Smith) by ip injection.

Drawing Description Text (18):
FIG. 17 is a graph showing the results of in vivo testing of MBI-11E3CN against S. aureus (Smith). Formulated peptide at various concentrations is administered by ip injection one hour after infection with S. aureus (Smith) by ip injection.

Drawing Description Text (19):
FIG. 18 is a graph showing the results of in vivo testing of: MBI-11F3CN against S. aureus (Smith). Formulated peptide at various concentrations is administered by ip injection one hour after infection with S. aureus (Smith) by ip injection.

Drawing Description Text (20):
FIG. 19 is a graph showing the results of in vivo testing of MBI-11G2CN against S. aureus (Smith). Formulated peptide at various concentrations is administered by ip injection one hour after infection with S. aureus (Smith) by ip injection.

Drawing Description Text (21):
FIG. 20 is a graph showing the results of in vivo testing of MBI-11CN against S. aureus (Smith). Formulated peptide at various concentrations is administered by ip injection one hour after infection with S. aureus (Smith) by ip injection.

Drawing Description Text (22):
FIG. 21 is a graph showing the results of in vivo testing of MBI-11B1CN against S. aureus (Smith). Formulated peptide at various concentrations is administered by ip injection one hour after infection with S. aureus (Smith) by ip injection.

Drawing Description Text (23):
FIG. 22 is a graph showing the results of in vivo testing of MBI-11B7CN against S. aureus (Smith). Formulated peptide at various concentrations is administered by ip injection one hour after infection with S. aureus (Smith) by ip injection.

Drawing Description Text (24):
FIG. 23 is a graph showing the results of in vivo testing of MBI-11B8CN against S. aureus (Smith). Formulated peptide at various concentrations is administered by ip injection one hour after infection with S. aureus (Smith) by ip injection.

Drawing Description Text (25):
FIG. 24 is a graph showing the results of in vivo testing of MBI-11G4CN against S. aureus (Smith). Formulated peptide at various concentrations is administered by ip injection one hour after infection with S. aureus (Smith) by ip injection.

Drawing Description Text (26):
FIGS. 25A and B display a graph showing the number of animals surviving an S. epidermidis infection after intravenous injection of MBI 10CN, gentamicin, or vehicle. Panel A, i.v. injection 15 min post-infection; panel B, i.v. injection 60 min post-infection.

Drawing Description Text (27):
FIG. 26 is a graph showing the number of animals surviving an MRSA infection mice after intravenous injection of MBI 11CN, gentamicin, or vehicle.

Detailed Description Text (5):
As used herein, a "peptide analogue", "analogue", or "variant" of indolicidin is at least 5 amino acids in length, has at least one basic amino acid (e.g., arginine and lysine) and has anti-microbial activity. Unless otherwise indicated, a named amino acid refers to the L-form. Basic amino acids include arginine, lysine, and derivatives. Hydrophobic residues include tryptophan, phenylalanine, isoleucine, leucine, valine, and derivatives.

Detailed Description Text (20):
Peptide analogues may alternatively be synthesized by recombinant production (see e.g., U.S. Pat. No. 5,593,866). A variety of host systems are suitable for production of the peptide analogues, including bacteria (e.g., E. coli), yeast (e.g., Saccharomyces cerevisiae), insect (e.g., Sf9), and mammalian cells (e.g., CHO, COS-7). Many expression vectors have been developed and are available for each of these hosts. Generally, bacteria cells and vectors that are functional in bacteria are used in this invention. However, at times, it may be preferable to have vectors that are functional in other hosts. Vectors and procedures for cloning and expression in E. coli are discussed herein and, for example, in Sambrook et al. (Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. 1987) and in Ausubel et al. (Current Protocols in Molecular Biology, Greene Publishing Co., 1995).

Detailed Description Text (21):
A DNA sequence encoding one or more indolicidin analogues is introduced into an expression vector appropriate for the host. In preferred embodiments, the analogue gene is cloned into a vector to create a fusion protein. The fusion partner is chosen to contain an anionic region, such that a bacterial host is protected from the toxic effect of the peptide. This protective region effectively neutralizes the antimicrobial effects of the peptide and also may prevent peptide degradation by host proteases. The fusion partner (carrier protein) of the invention may further function to transport the fusion peptide to inclusion bodies, the periplasm, the outer membrane, or the extracellular environment. Carrier proteins suitable in the context of this invention specifically include, but are not limited to, glutathione-S-transferase (GST), protein A from Staphylococcus aureus, two synthetic IgG-binding domains (ZZ) of protein A, outer membrane protein F, .beta.-galactosidase (lacZ), and various products of bacteriophage .lambda. and bacteriophage T7. From the teachings provided herein, it is apparent that other proteins may be used as carriers. Furthermore, the entire carrier protein need not be used, as long as the protective anionic region is present. To facilitate isolation of the peptide sequence, amino acids susceptible to chemical cleavage (e.g., CNBr) or enzymatic cleavage (e.g., V8 protease, trypsin) are used to bridge the peptide and fusion partner. For expression in E. coli, the fusion partner is preferably a normal intracellular protein that directs expression toward inclusion body formation. In such a case, following cleavage to release the final product, there is no requirement for renaturation of the peptide. In the present invention, the DNA cassette, comprising fusion partner and peptide gene, may be inserted into an expression vector, which can be a plasmid, virus or other vehicle known in the art. Preferably, the expression vector is a plasmid that contains an inducible or constitutive promoter to facilitate the efficient transcription of the inserted DNA sequence in the host. Transformation of the host cell with the recombinant DNA may be carried out by Ca.sup.++ -mediated techniques, by electroporation, or other methods well known to those skilled in the art.

Detailed Description Text (25):
Within a preferred embodiment, the vector is capable of replication in bacterial cells. Thus, the vector may contain a bacterial origin of replication. Preferred bacterial origins of replication include f1-ori and col E1 ori, especially the ori derived from pUC plasmids. Low copy number vectors (e.g., pPD100) may also be used, especially when the product is deleterious to the host.

Detailed Description Text (26):
The plasmids also preferably include at least one selectable marker that is functional in the host. A selectable marker gene confers a phenotype on the host that allows transformed cells to be identified and/or selectively grown. Suitable selectable marker genes for bacterial hosts include the chloroamphenicol resistance

gene (Cm.sup.r), ampicillin resistance gene (Amp.sup.r), tetracycline resistance gene (Tc.sup.r) kanamycin resistance gene (Kan.sup.r), and others known in the art. To function in selection, some markers may require a complementary deficiency in the host.

Detailed Description Text (28):
The vector may also contain a gene coding for a repressor protein, which is capable of repressing the transcription of a promoter that contains a repressor binding site. Altering the physiological conditions of the cell can depress the promoter. For example, a molecule may be added that competitively binds the repressor, or the temperature of the growth media may be altered. Repressor proteins include, but are not limited to the E. coli lacI repressor (responsive to induction by IPTG), the temperature sensitive .lambda.cI857 repressor, and the like.

Detailed Description Text (38):
Monoclonal antibodies may also be readily generated from hybridoma cell lines using conventional techniques (see U.S. Pat. No. RE 32,011, U.S. Pat. Nos. 4,902,614, 4,543,439, and 4,411,993; see also Antibodies: A Laboratory Manual, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988). Briefly, within one embodiment, a subject animal such as a rat or mouse is injected with peptide, generally administered as an emulsion in an adjuvant such as Freund's complete or incomplete adjuvant in order to increase the immune response. The animal is generally boosted at least once prior to harvest of spleen and/or lymph nodes and immortalization of those cells. Various immortalization techniques, such as mediated by Epstein-Barr virus or fusion to produce a hybridoma, may be used. In a preferred embodiment, immortilization occurs by fusion with a suitable myeloma cell line to create a hybridoma that secretes monoclonal antibody. Suitable myeloma lines include, for example, NS-1 (ATCC No. TIB 18), and P3X63-Ag 8.653 (ATCC No. CRL 1580). The preferred fusion partners do not express endogenous antibody genes. After about seven days, the hybridomas may be screened for the presence of antibodies that are reactive against a telomerase protein. A wide variety of assays may be utilized (see Antibodies: A Laboratory Manual, Harlow and Lane (eds.). Cold Spring Harbor Laboratory Press, 1988).

Detailed Description Text (45):
The selected analogues may be further tested for their toxicity to normal mammalian cells. An exemplary assay is a red blood cell (RBC) (erythrocyte) hemolysis assay. Briefly, red blood cells are isolated from whole blood, typically by centrifugation, and washed free of plasma components. A 1% (v/v) suspension of erythrocytes in isotonic saline is incubated with different concentrations of peptide analogue. Generally, the analogue will be in a suitable formulation buffer. After incubation for approximately 1 hour at 37.degree. C., the cells are centrifuged, and the absorbance of the supernatant at 540 nm is determined. A relative measure of lysis is determined by comparison to absorbance after complete lysis of erythrocytes using NH.sub.4 Cl or equivalent (establishing a 100% value). An analogue that is not lytic, or is only moderately lytic, as exemplified in Example 8, is desirable and is suitable for further screening. Other in vitro toxicity assays, for example measurement of toxicity towards cultured mammalian cells, may be used to assess in vitro toxicity.

Detailed Description Text (50):
The antibiotic activity of selected analogues may be assessed in vivo for their ability to ameliorate microbial infections using animal models. Within these assays, an analogue is useful as a therapeutic if inhibition of microorganismal growth compared to inhibition with vehicle alone is statistically significant. This measurement can be made directly from cultures isolated from body fluids or sites, or indirectly, by assessing survival rates of infected animals. For assessment of antibacterial activity several animal models are available, such as acute infection models including those in which (a) normal mice receive a lethal dose of microorganisms, (b) neutropenic mice receive a lethal dose of microorganisms or (c) rabbits receive an inoculum in the heart, and chronic infection models. The model selected will depend in part on the intended clinical indication of the analogue.

Detailed Description Text (51):
By way of example, in one such normal mouse model, mice are inoculated ip or iv with

a lethal dose of bacteria. Typically, the dose is such that 90-100% of animals die within 2 days. The choice of a microrganismal strain for this assay depends, in part, upon the intended application of the analogue, and in the accompanying examples, assays are carried out with three different Staphylococcus strains. Briefly, shortly before or after inoculation (generally within 60 minutes), analogue in a suitable formulation buffer is injected. Multiple injections of analogue may be administered. Animals are observed for up to 8 days post-infection and the survival of animals is recorded. Successful treatment either rescues animals from death or delays death to a statistically significant level, as compared with non-treatment control animals. Analogues that show better efficacy than indolicidin itself are preferred.

Detailed Description Text (88):
As noted above, the present invention provides methods for treating and preventing infections by administering to a patient a therapeutically effective amount of a peptide analogue of indolicidin as described herein. Patients suitable for such treatment may be identified by well-established hallmarks of an infection, such as fever, pus, culture of organisms, and the like. Infections that may be treated with peptide analogues include those caused by or due to microorganisms. Examples of microorganisms include bacteria (e.g., Gram-positive, Gram-negative), fungi, (e.g., yeast and molds), parasites (e.g., protozoans, nematodes, cestodes and trematodes), viruses, and prions. Specific organisms in these classes are well known (see for example, Davis et al., Microbiology, 3.sup.rd edition, Harper & Row, 1980). Infections include, but are not limited to, toxic shock syndrome, diphtheria, cholera, typhus, meningitis, whooping cough, botulism, tetanus, pyogenic infections, dysentery, gastroenteritis, anthrax, Lyme disease, syphilis, rubella, septicemia and plague.

Detailed Description Text (89):
Effective treatment of infection may be examined in several different ways. The patient may exhibit reduced fever, reduced number of organisms, lower level of inflammatory molecules (e.g., IFN-.gamma., IL-12, IL-1, TNF), and the like.

Detailed Description Text (96):
Pharmaceutical compositions of the present invention are administered in a manner appropriate to the infection or disease to be treated. The amount and frequency of administration will be determined by factors such as the condition of the patient, the cause of the infection, and the severity of the infection. Appropriate dosages may be determined by clinical trials, but will generally range from about 0.1 to 50 mg/kg.

Detailed Description Text (99):
The analogues, especially the labeled analogues, may be used in image analysis and diagnostic assays or for targeting sites in eukaryotic multicellular and single cell cellular organisms and in prokaryotes. As a targeting system, the analogues may be coupled with other peptides, proteins, nucleic acids, antibodies and the like.

Detailed Description Text (119):
Peptide analogues are alternatively produced by recombinant DNA technique in bacterial host cells. The peptide is produced as a fusion protein, chosen to assist in transporting the fusion peptide to inclusion bodies, periplasm, outer membrane or extracellular environment.

Detailed Description Text (123):
The plasmid pR2h-11, employing a T7 promoter, high copy origin of replication. Ap.sup.r marker and containing the gene of the fusion protein, is co-electroporated with pGP1-2 into E. coli strain XL1-Blue. Plasmid pGP1-2 contains a T7 RNA polymerase gene under control of a lambda promoter and cI857 repressor gene. Fusion protein expression is induced by a temperature shift from 30.degree. C. to 42.degree. C. Inclusion bodies are washed with solution containing solubilizer and extracted with organic extraction solvent. Profiles of the samples are analyzed by SDS-PAGE. FIG. 1 shows the SDS-PAGE analysis and an extraction profile of inclusion body from whole cell. The major contaminant in the organic solvent extracted material is .beta.-lactamase (FIG. 1). The expression level in these cells is presented in Table 4.

Detailed Description Text (124):
In addition, a low-copy-number vector, pPD100, which contains a chloramphenicol resistance gene, is used to express MBI-11 in order to eliminate the need for using ampicillin, thereby reducing the appearance of .beta.-lactamase in extracted material. This plasmid allows selective gene expression and high-level protein overproduction in E. coli using the bacteriophage T7 RNA polymerase/T7 promoter system (Dersch et al., FEMS Microbiol. Lett. 123: 19-26, 1994). pPD100 contains a chloramphenicol resistance gene (CAT) as a selective marker, a multiple cloning site, and an ori sequence derived from the low-copy-number vector pSC101. There are only about 4 to 6 copies of these plasmids per host cell. The resulting construct containing MBI-11 is called pPDR2h-11. FIG. 2 presents a gel electrophoresis analysis of the MBI-11 fusion protein expressed in this vector. Expression level of MBI-11 fusion protein is comparable with that obtained from plasmid pR2h-11. The CAT gene product is not apparent, presumably due to the low-copy-number nature of this plasmid, CAT protein is not expressed at high levels in pPDR2h-11.

Detailed Description Text (168):
Using this assay, peptide activity, measured by dye release, is shown to be extremely sensitive to the composition of the liposomes at many liposome to peptide ratios (L/P) (FIG. 7). Specifically, addition of cholesterol to liposomes composed of egg phosphotidylcholine (PC) virtually abolishes membrane permeabilizing activity of MBI 11CN, even at very high lipid to peptide molar ratios (compare with egg PC liposomes containing no cholesterol). This in vitro selectivity may mimic that observed in vitro for bacterial cells in the presence of mammalian cells.

Detailed Description Text (171):
An alternative method for measuring peptide-membrane interaction uses the E.coli strain ML-35 (Lehrer et al., J. Clin. Invest., 84: 553, 1989), which contains a chromosomal copy of the lacZ gene encoding .beta.-galactosidase and is permease deficient. This strain is used to measure the effect of peptide on the inner membrane through release of .beta.-galactosidase into the periplasm. Release of .beta.-galactosidase is measured by spectrophotometrically monitoring the hydrolysis of its substrate o-nitrophenol .beta.-D-galactopyranoside (ONPG). The maximum rate of hydrolysis (V.sub.max) is determined for aliquots of cells taken at various growth points.

Detailed Description Text (172):
A preliminary experiment to determine the concentration of peptide required for maximal activity against mid-log cells, diluted to 4.times.10.sup.7 CFU/ml, yields a value of 50 .mu.g/ml, which is used in all subsequent experiments. Cells are grown in two different growth media, Terrific broth (TB) and Luria broth (LB) and equivalent amounts of cells are assayed during their growth cycles. The resulting activity profile of MBI 11B7CN is shown in FIG. 8. For cells grown in the enriched TB media, maximum activity occurs at early mid-log (140 min), whereas for cells grown in LB media, the maximum occurs at late mid-log (230 min). Additionally, only in LB, a dip in activity is observed at 140 min. This drop in activity may be related to a transition in metabolism, such as a requirement for utilization of a new energy source due to depletion of the original source, which does not occur in the more enriched TB media. A consequence of a metabolism switch would be changes in the membrane potential.

Detailed Description Text (173):
To test whether membrane potential has an effect on peptide activity, the effect of disrupting the electrochemical gradient using the potassium ionophore valinomycin is examined. Cells pre-incubated with valinomycin are treated with peptide and for MBI 10CN and MBI 11CN ONPG hydrolysis diminished by approximately 50% compared to no pre-incubation with valinomycin (FIG. 9). Another cationic peptide that is not sensitive to valinomycin is used as a positive control.

Detailed Description Text (177):
A red blood cell (RBC) lysis assay is used to group peptides according to their ability to lyse RBC under standardized conditions compared with MBI 11CN and Gramicidin-S. Peptide samples and washed sheep RBC are prepared in isotonic saline with the final pH adjusted to between 6 and 7. Peptide samples and RBC suspension

are mixed together to yield solutions that are 1% (v/v) RBC and 5, 50 or 500 .mu.g/ml peptide. Assay mixtures are incubated for 1 hour at 37.degree. C. with constant shaking, centrifuged, and the supernatant is measured for absorbance at 540 nm, which detects released hemoglobin. The percentage of released hemoglobin is determined by comparison with a set of known standards lysed in water. Each set of assays also includes MBI 11CN (500 .mu.g/ml) and Gramicidin-S (5 .mu.g/ml) as "low lysis" and "high lysis" controls, respectively.

Detailed Description Text (185):
The in vitro lifetime of free peptide analogues in plasma and in blood is determined by measuring the amount of peptide present after set incubation times. Blood is collected from sheep, treated with an anticoagulant (not heparin) and, for plasma preparation, centrifuged to remove cells. Formulated peptide is added to either the plasma fraction or to whole blood and incubated. Following incubation, peptide is identified and quantified directly by reversed phase HPLC. Extraction is not required as the free peptide peak does not overlie any peaks from blood or plasma.

Detailed Description Text (187):
Peptide levels in plasma in vivo are measured after iv or ip administration of 80-100% of the maximum tolerated dose of peptide analogue in either formulation C1 or D. MBI 11CN in formulation C1 is injected intravenously into the tail vein of CD1 ICRBR strain mice. At various times post-injection, mice are anesthetized and blood is drawn by cardiac puncture. Blood from individual mice is centrifuged to separate plasma from cells. Plasma is then analyzed by reversed phase HPLC column. The resulting elution profiles are analyzed for free peptide content by UV absorbance at 280 nm, and these data are converted to concentrations in blood based upon a calibrated standard. Each data point represents the average blood level from two mice. In this assay, the detection limit is approximately 1 .mu.g/ml, less than 3% of the dose administered.

Detailed Description Text (205):
Analogues are tested for their ability to rescue mice from lethal bacterial infections. The animal model used is an intraperitoneal (ip) inoculation of mice with 10.sup.6 -10.sup.8 Gram-positive organisms with subsequent administration of peptide. The three pathogens investigated, methicillin-sensitive S. aureus (MSSA), methicillin-resistant S. aureus (MRSA), or S. epidermidis are injected ip into mice. For untreated mice, death occurs within 12-18 hours with MSSA and S. epidermis and within 6-10 hours with MRSA.

Detailed Description Text (206):
Peptide is administered by two routes, intraperitoneally, at one hour post-infection, or intravenously, with single or multiple doses given at various times pre- and post-infection.

Detailed Description Text (207):
MSSA infection. In a typical protocol, groups of 10 mice are infected intraperitoneally with a LD.sub.90-100 dose (5.2.times.10.sup.6 CFU/mouse) of MSSA (Smith, ATCC # 19640) injected in brain-heart infusion containing 5% mucin. This strain of S. aureus is not resistant to any common antibiotics. At 60 minutes post-infection, MBI 10CN or MBI 11CN. in formulation D, is injected intraperitoneally at the stated dose levels. An injection of formulation alone serves as a negative control and administration of ampicillin serves as a positive control. The survival of the mice is monitored at 1, 2, 3 and 4 hrs post-infection and twice daily thereafter for a total of 8 days.

Detailed Description Text (208):
As shown in FIG. 14, MBI 10CN is maximally active against MSSA (70-80% survival) at doses of 14.5 to 38.0 mg/kg, although 100% survival is not achieved. Below 14.5 mg/kg, there is clear dose-dependent survival. At these lower dose levels, there appears to be an animal-dependent threshold, as the mice either die by day 2 or survive for the full eight day period. As seen in FIG. 15. MBI 11CN, on the other hand, rescued 100% of the mice from MSSA infection at a dose level of 35.7 mg/kg, and was therefore as effective as ampicillin. There was little or no activity at any of the lower dose levels, which indicates that a minimum bloodstream peptide level must be achieved during the time that bacteria are a danger to the host.

Detailed Description Text (211):
S. epidermidis infection. Peptide analogues generally have lower MIC values against S. epidermidis in vitro, therefore, lower blood peptide levels might be more effective against infection.

Detailed Description Text (212):
In a typical protocol, groups of 10 mice are injected intraperitoneally with an LD.sub.90-100 dose (2.0.times.10.sup.8 CFU/mouse) of S. epidermidis (ATCC # 12228) in brain-heart infusion both containing 5% mucin. This strain of S. epidermidis is 90% lethal after 5 days. At 15 mins and 60 mins post-infection, various doses of MBI 11CN in formulation D are injected intravenously via the tail vein. An injection of formulation only serves as the negative control and injection of gentamicin serves as the positive control, both are injected at 60 minutes post-infection. The survival of the mice is monitored at 1, 2, 3, 4, 6 and 8 hrs post-infection and twice daily thereafter for a total of 8 days.

Detailed Description Text (213):
As shown in FIGS. 25A and 25B, MBI 11CN prolongs the survival of the mice. Efficacy is observed at all three dose levels with treatment 15 minutes post-infection, however, there is less activity at 30 minutes post-infection and no significant effect at 60 minutes post-infection. Time of administration appears to be important in this model system, with a single injection of 6.1 mg/kg 15 minutes post-infection giving the best survival rate.

Detailed Description Text (214):
MRSA infection. MRSA infection, while lethal in a short period of time, requires a much higher bacterial load than MSSA. In a typical protocol, groups of 10 mice are injected intraperitoneally with a LD.sub.90-100 dose (4.2.times.10.sup.7 CFU/mouse) of MRSA (ATCC # 33591) in brain-heart infusion containing 5% mucin. The treatment protocols are as follows, with the treatment times relative to the time of infection:

Detailed Description Text (215):
MBI 11CN is injected intravenously in the tail vein in formulation D. Survival of mice is recorded at 1, 2, 3, 4, 6, 8, 10, 12, 20, 24 and 30 hrs post-infection and twice daily thereafter for a total of 8 days. There was no change in the number of surviving mice after 24 hrs (FIG. 26).

Detailed Description Text (216):
The 1 mg/kg (20 min) treatment protocol, with injections 5 minutes apart centered on the infection time, delayed the death of the mice to a significant extent with one survivor remaining at the end of the study. The results presented in Table 19 suggest that a sufficiently high level of MBI 11CN maintained over a longer time period would increase the number of mice surviving. The 5 mg/kg and 1 mg/kg (2 hr) results, where there is no improvement in survivability over the negative control, indicates that injections 1 hour apart, even at a higher level, are not effective against MRSA.

Detailed Description Text (219):
A solution of 2% (w/w) polysorbate 80 is prepared in water and placed in a suitable reaction vessel, such as a quartz cell. Other containers that are UV translucent or even opaque can be used if provision is made for a clear light path or an extended reaction time. In addition, the vessel should allow the exchange of air but minimize evaporation.

Detailed Description Paragraph Table (1):
TABLE 1 Class of Antibiotic Antibiotic Mode of Action PENICILLINS Blocks the formation of new cell walls in bacteria Natural Penicillin G, Benzylpenicillin Penicillin V, Phenoxymethylpenicillin Penicillinase resistant Methicillin, Nafcillin, Oxacillin Cloxacillin, Dicloxacillin Acylamino-penicillins Ampicillin, Amoxicillin Carboxy-penicillins Ticarcillin, Carbenicillin Ureido-penicillins Mezlocillin, Azlocillin, Piperacillin CARBAPENEMS Imipenem, Meropenem Blocks the formation of new cell walls in bacteria MONOBACTAMS Aztreonam Blocks the formation of new cell walls in bacteria CEPHALOSPORINS Prevents formation of new cell walls in

bacteria 1st Generation Cephalothin, Cefazolin 2nd Generation Cefaclor, Cefamandole Cefuroxime, Cefonicid, Cefmetazole, Cefotetan, Cefprozil 3rd Generation Cefetamet, Cefoperazone Cefotaxime, Ceftizoxime Ceftriaxone, Ceftazidime Cefixime, Cefpodoxime, Cefsulodin 4th Generation Cefepime CARBACEPHEMS Loracarbef Prevents formation of new cell walls in bacteria CEPHAMYCINS Cefoxitin Prevents formation of new cell walls in bacteria QUINOLONES Fleroxacin, Nalidixic Acid Inhibits bacterial DNA Norfloxacin, Ciprofloxacin synthesis Ofloxacin, Enoxacin Lomefloxacin, Cinoxacin TETRACYCLINES Doxycycline, Minocycline, Inhibits bacterial protein Tetracycline synthesis, binds to 30S ribosome subunit. AMINOGLYCOSIDES Amikacin, Gentamicin, Kanamycin, Inhibits bacterial protein Netilmicin, Tobramycin, synthesis, binds to 30S Streptomycin ribosome subunit. MACROLIDES Azithromycin, Clarithromycin, Inhibits bacterial protein Erythromycin synthesis, binds to 50S ribosome subunit Derivatives of Erythromycin estolate, Erythromycin Erythromycin stearate Erythromycin ethylsuccinate Erythromycin gluceptate Erythromycin lactobionate GLYCOPEPTIDES Vancomycin, Teicoplanin Inhibits cell wall synthesis, prevents peptidoglycan elongation. MISCELLANEOUS Chloramphenicol Inhibits bacterial protein synthesis, binds to 50S ribosome subunit. Clindamycin Inhibits bacterial protein synthesis, binds to 50S ribosome subunit. Trimethoprim Inhibits the enzyme dihydrofolate reductase, which activates folic acid. Sulfamethoxazole Acts as antimetabolite of PABA & inhibits synthesis of folic acid Nitrofurantoin Action unknown, but is concentrated in urine where it can act on urinary tract bacteria Rifampin Inhibits bacterial RNA polymerase Mupirocin Inhibits bacterial protein synthesis

Detailed Description Paragraph Table (4):
TABLE 4 % protein Fusion Mol.mass in whole % in inclusion % which is protein (kDa)
cell lysate body extract MBI-11 peptide MBI-11 20.1 15 42 7.2

Detailed Description Paragraph Table (22):
TABLE 19 Time of Observation Percentage of Animals Surviving (Hours post-infection)
No Treatment Treatment 6 50% 70% 8 0 40% 10 0 30% 12 0 20%

WEST

Generate Collection Print

over 80 pgs

L7: Entry 13 of 20 File: USPT May 14, 2002

DOCUMENT-IDENTIFIER: US 6388056 B1
TITLE: Inhibition of transglutaminase-mediated microbial interaction with a mammalian host

Abstract Text (1):
The infection of a mammalian host by a microorganism can be prevented or treated through the administration of substrates for transglutaminases or antibodies against such substrates that inhibit the transglutaminase-mediated interaction of the microorganism with the mammalian host. These compounds may be used in the identification, prevention or treatment of microbial infection of mammalian hosts such as immunocompromised or immunosuppressed humans, for example, those having AIDS or undergoing transplantation or anti-cancer therapy.

Brief Summary Text (2):
The present invention relates to compounds and methods for the prevention and treatment of microbial infection of a mammalian host through the administration of substrates for transglutaminases or antibodies against such substrates that inhibit the transglutaminase-mediated interaction of the microorganism with the mammalian host. These compounds and methods may be used preferably in the identification, prevention or treatment of microbial infection of mammalian hosts such as immunocompromised or immunosuppressed humans, for example, those having AIDS or undergoing transplantation or anti-cancer therapy.

Brief Summary Text (4):
Whether pathogenic or opportunistic, microorganisms have evolved numerous mechanisms to facilitate their establishment and proliferation in mammalian hosts. During initial infection, the interaction of a microorganism with its mammalian host can include attachment or adhesion to the host cell surface, invasion of host cells, and elaboration of toxins, for example. In certain instances, this interaction can be nonspecific. In others, such microbial interaction involves the specific binding of the microorganism to a particular receptor or receptor complex expressed on the host cell surface. In turn, the binding event can trigger changes in the microorganism and/or the mammalian host cell, leading to the progression of infection.

Brief Summary Text (5):
The host cell functions of molecules involved in certain microbial interaction are unknown in some cases and known in others. Mammalian transglutaminases are examples of those in the latter category for which the molecular mechanism of action and/or role in host cell growth or development has been elucidated. In general, transglutaminases are enzymes that catalyze intermolecular crosslinks by the formation of highly stable isodipeptide bonds between the .gamma.-carbonyl group of glutamine and the .epsilon.-amino group of lysine residues, which are resistant to proteases, sodium dodecyl sulfate and heat. Epithelial cell transglutaminases are important for the formation of cornified envelopes of mature squamous epithelial cells.

Brief Summary Text (6):
Only recently have investigators shown that certain microorganisms may express proteins capable of acting as substrates for, and thus interact with, mammalian transglutaminases. One example is hyphal wall protein 1 (Hwp1), which is expressed on hyphal surfaces of the pathogenic fungus, Candida albicans. Hwp1 consists of an N-terminal proline and glutamine-rich repetitive amino acid sequence that is exposed

on the hyphal surface, and a cell wall-anchored serine and threonine-rich C-terminus. The composition of the N-terminal amino acid repeats is reminiscent of mammalian transglutaminase substrates. It is now known that Hwp1 can serve as a substrate in transglutaminase-mediated cross-linking reactions.

Brief Summary Text (7):
Candida is an ubiquitous yeast recognized as the causative agent of candidiasis (Candida mycosis). At least 90% of the disorders are caused by the species C. albicans, which is an opportunistic yeast able only to elicit mild superficial infections in normal individuals. Fungal infections associated with severe infections of the mucous membrane and with invasive infections of individual organs are observed ever more frequently as a result of the increasing number of patients with immune defense weakness, e.g., patients with acquired immunodeficiency syndrome (AIDS) or patients undergoing immunosuppressive therapy.

Brief Summary Text (8):
If left untreated, such systemic infections frequently lead to the death of the patients. At present, the treatment for invasive infections is based on relatively few antimycotics, such as amphotericin B and flucytosine, or the azole derivatives fluconazole and itraconazole. These antimycotics cause serious, sometimes different, side effects, such as renal insufficiency, hypocalcemia and anemia, as well as unpleasant constitutional symptoms such as fever, shivering and low blood pressure.

Brief Summary Text (10):
Oropharyngeal and esophageal candidiasis are among the most frequent opportunistic fungal infections observed in human immunodeficiency virus positive (HIV+) and AIDS patients, occurring in the majority of patients. The pathogenesis is complex and is thought to involve multiple host factors that include loss of cell mediated immunity and altered phagocytic cell activity. The current status of the AIDS epidemic is one of increasing numbers of individuals infected and no cure. Many infected individuals may live for a long time with HIV in an essentially permanent immunocompromised state. Because of the loss of the cellular component of the immune system, AIDS patients are susceptible to invasion of submucosal tissue by C. albicans. The frequency of candidal infections may also be a result of the prophylactic use of antibacterial drugs used in AIDS patients to minimize other opportunistic infections. Candidal infections increase in severity and recur more frequently as the immunodeficiency progresses.

Brief Summary Text (11):
While treatment with antifungal drugs can be effective, the increasing frequency of resistant strains of C. albicans, and the systemic side effects of the drugs prompts exploration of novel strategies to interrupt the sequence of events leading to disease and to expand the repertoire of antifungal drugs. An antifungal strategy based on biological interactions between C. albicans and the oral mucosa would be of great benefit to those with such fungal infections, e.g., patients with long-term immunodeficiencies.

Brief Summary Text (13):
A feature of C. albicans growth that is correlated with pathogenicity in the oral cavity is the ability to transform from budding to filament-extending growth. Filamentous forms adhere more readily to buccal epithelial cells than budding yeasts, and histologically are a prominent feature of invasion of the mucosa. Knowledge of the molecular events that transform C. albicans to the pathogenic filamentous form as well as detailed investigations of the hyphal surface at the molecular level are necessary for understanding the pathogenesis of oral candidiasis.

Brief Summary Text (15):
In the oral mucosa, Hwp1 adherence may help C. albicans resist the mechanical forces that clear the oral mucosa, enhancing colonization. Hwp1-mediated stabilized adhesion may induce accelerated maturation of epithelial cells, partially explaining the association of candidiasis with increased turnover of basal keratinocytes. Alternatively, Hwp1 may be important for interacting with host transglutaminases other than those associated with epithelial cells. Hwp1 may also be important for the spatial expression of other pathogenically-important proteins on the germ tube

surface.

Brief Summary Text (16):
A valuable contribution to the art therefore would be compounds and methods for the prevention and treatment of microbial infection of a mammalian host through the administration of substrates for transglutaminases or antibodies against such substrates that inhibit the transglutaminase-mediated interaction of the microorganism with the mammalian host. These compounds and methods may be used preferably in the identification, prevention or treatment of microbial infection of mammalian hosts such as immunocompromised humans having AIDS.

Brief Summary Text (18):
Accordingly, an objective of the present invention includes compounds and methods for the prevention and treatment of a microbial infection of a mammalian host through the administration of substrates for transglutaminases or antibodies against such substrates that inhibit the transglutaminase-mediated interaction of the microorganism with the mammalian host.

Brief Summary Text (19):
Another objective pertains to compounds and methods for the identification, prevention or treatment of microbial infection of mammalian hosts such as immunocompromised humans having AIDS. Yet another objective relates to the identification of the absence or presence of microbial infection and the site(s) of such infection. A further objective is a diagnostic kit for such identification. One other objective of the present invention is the prevention and treatment of a microbial infection of a mammalian host through gene therapy, whereby host cells, for example, are engineered to express substrates for transglutaminases that inhibit the transglutaminase-mediated interaction of the microorganism with the mammalian host. These and other objectives are achieved through the following preferred embodiments.

Brief Summary Text (20):
One aspect of the invention is a purified polypeptide comprising the amino acid sequence of SEQ. ID NO. 1, wherein said polypeptide is capable of acting as a substrate for mammalian transglutaminases. Another aspect is an isolated DNA molecule encoding the polypeptide having the amino acid sequence of SEQ. ID NO. 1, and an isolated DNA molecule comprising the nucleotide sequence encoding the polypeptide of SEQ. ID NO. 1. A further aspect is a nucleic acid capable of hybridizing under high stringency conditions to the DNA molecule of an isolated DNA molecule comprising the nucleotide sequence encoding the polypeptide of SEQ. ID NO. 1. In addition, an aspect of the invention is a vector comprising DNA encoding the polypeptide of SEQ. ID NO. 1, a host cell transformed with that vector, and that transformed host cell which produces a protein capable of acting as a substrate for mammalian transglutaminases.

Brief Summary Text (21):
An aspect of the present invention is an isolated antibody against the polypeptide comprising the amino acid sequence of SEQ. ID NO. 1 (or an antigenic portion thereof), wherein said polypeptide (or an antigenic portion thereof) is capable of acting as a substrate for mammalian transglutaminases. In a preferred embodiment, the antibody is a monoclonal antibody. In another preferred embodiment, the antibody is capable of inhibiting the interaction of a microorganism with a mammalian cell, preferably where the microorganism is a bacteria or yeast, and more preferably where the microorganism is C. albicans. In one other preferred embodiment of the antibody, the mammalian cell is a human cell, preferably an epithelial cell, more preferably a mucosal epithelial cell, and most preferably a buccal epithelial cell.

Brief Summary Text (22):
Another aspect of the invention is a method of preventing or treating infection by a microorganism of a mammalian host comprising the steps of administering to said host an effective amount of purified Hwp1 protein, antibody against said Hwp1 protein, or polypeptide comprising the amino acid sequence of SEQ. ID NO. 1, wherein said polypeptide is capable of acting as a substrate for mammalian transglutaminases, antibody against said polypeptide, purified proline-rich protein, or antibody against said proline-rich protein, in a pharmaceutically acceptable sterile vehicle,

and inhibiting the interaction of said microorganism with the cells of said host. In another preferred embodiment, the antibody is capable of inhibiting the interaction of a microorganism with a mammalian cell, preferably where the microorganism is a bacteria or yeast, and more preferably where the microorganism is C. albicans. In one other preferred embodiment, the mammalian cell is a human cell, preferably an epithelial cell, more preferably a mucosal epithelial cell, and most preferably a buccal epithelial cell. In yet another preferred embodiment, the administering is performed orally. In a preferred embodiment, the mammalian host is immunocompromised, and in another, the infection is associated with AIDS.

Brief Summary Text (23):
Another aspect of the invention is a vaccine for preventing infection by a microorganism of a mammalian host comprising an effective amount of purified Hwp1 protein, antibody against said Hwp1 protein, or polypeptide comprising the amino acid sequence of SEQ. ID NO. 1, wherein said polypeptide is capable of acting as a substrate for mammalian transglutaminases, antibody against said polypeptide, purified proline-rich protein, or antibody against said proline-rich protein, in a pharmaceutically acceptable sterile vehicle, wherein said vaccine is capable of inhibiting the interaction of said microorganism with the cells of said host. In another preferred embodiment, the vaccine is capable of inhibiting the interaction of a microorganism with a mammalian cell, preferably where the microorganism is a bacteria or yeast, and more preferably where the microorganism is C. albicans. In one other preferred embodiment, the mammalian cell is a human cell, preferably an epithelial cell, more preferably a mucosal epithelial cell, and most preferably a buccal epithelial cell. In yet another preferred embodiment, the administering is performed orally. In a preferred embodiment, the mammalian host is immunocompromised, and in another, the infection is associated with AIDS.

Brief Summary Text (26):
Yet another aspect of the invention is a method of preventing or treating infection by a microorganism of a mammalian host, comprising the steps of administering syngeneic host cells transformed with the vector comprising DNA encoding the polypeptide of SEQ. ID NO. 1, wherein said transformed syngeneic host cells produce a protein capable of acting as a substrate for mammalian transglutaminases and inhibiting the interaction of said microorganism with the cells of said host. In a preferred embodiment, the transformed syngeneic host cells are capable of inhibiting the interaction of a microorganism with a mammalian cell, preferably where the microorganism is a bacteria or yeast, and more preferably where the microorganism is C. albicans. In one other preferred embodiment, the mammalian cell is a human cell, preferably an epithelial cell, more preferably a mucosal epithelial cell, and most preferably a buccal epithelial cell. In yet another preferred embodiment, the administering is performed orally. In a preferred embodiment, the mammalian host is immunocompromised, and in another, the infection is associated with AIDS.

Drawing Description Text (8):
FIG. 5 depicts adherence to human buccal epithelial cells (BECs) of heterozygous hwp1/HWP1 (CAH7) and homozygous hwp1/hwp1 (CAH7-1A) mutants, and HWP1 revertant (CAHR3) relative to the homozygous HWP1/HWP1 strain (UnoPP-1). The adherence of each strain relative to UnoPP-1 was determined. The values are the means .+-.SD of two experiments performed in duplicate. The Student's t test was used to determine statistically significant differences.

Detailed Description Text (2):
Transglutaminases are a family of enzymes that are Ca.sup.2+ dependent and have thiol-containing active sites that form covalent N.sup.68 -(.gamma.-glutamyl)lysine isodipeptide bonds that are stable to denaturants such as urea, SDS and reducing agents. In squamous epithelial cells, transglutaminases catalyze the formation of rigid, cornified envelopes forming an impenetrable, first line host defense barrier. Buccal epithelial cell (BEC) transglutaminases cross-link salivary proline-rich proteins (PRPs) to proteins on BEC surfaces in a process that may affect mucosal pellicle function and counteract microbial adhesion.

Detailed Description Text (3):
While conformational constraints exist for the endoglutamine substrate protein, essentially any primary amine, such as putrescine, methylamine, cadaverine or free

lysine can participate in cross-linking reactions. The conformational constraints within proteins that favor certain glutamine and lysine residues as substrates for cross-linking suggest a preference for terminal sequence regions, and/or sequences that are exposed on the protein surface. The envelope precursors that serve as substrates for epithelial cell transglutaminase are a heterogeneous group of proteins with conserved termini, including involucrin, cornifins, loricrin, small proline-rich proteins (SPRPs), for example.

Detailed Description Text (5):
APRPs, in combination with basic and neutral proline-rich proteins, constitute 70% of the proteins in parotid saliva. Multiple functions have been ascribed to APRPs that include detoxification of tannins, tooth remineralization, and serving as receptors for microorganisms. APRPs have proline and glutamine-rich regions. Certain APRPs are transglutaminase substrates and become covalently linked to BECs. The ability to cross-link APRPs to donor BECs from healthy adults suggests that the surface of oral epithelial squames is a partially denuded protein matrix replete with an associated transpeptidase which may cross-link appropriate substrates such as APRPs that come in contact with the cell surface. In addition, APRPs may function to resist stabilized adhesion of C. albicans by forming salivary pellicle and diminishing transglutaminase activity on the surfaces of oral squames.

Detailed Description Text (8):
Germ tubes and hyphae of C. albicans possess unique surface proteins that are not expressed in yeasts. Among these proteins is an outer mannoprotein, Hwp1, with a cell surface-exposed, ligand-binding domain at the N-terminus and with C-terminal features that confer covalent integration into the .beta.-glucan of the cell wall. The primary amino acid sequence of the Hwp1 N-terminal domain shows general similarity to substrates of mammalian transglutaminases in the abundance of glutamine and proline residues, consecutive glutamine residues, and short amino acid repeats.

Detailed Description Text (9):
In accordance with the present invention, a substrate for mammalian transglutaminases refers to any purified or synthetic compound (or fragment thereof) that binds (covalently or noncovalently) to one or more mammalian transglutaminases. In a preferred embodiment, a substrate for mammalian transglutaminases can inhibit the binding of one or more mammalian transglutaminases to purified Hwp1 protein or a polypeptide comprising the amino acid sequence of SEQ. ID NO. 1 (FIG. 1), wherein said polypeptide is itself capable of acting as a substrate for mammalian transglutaminases. In addition, microbial interaction with a mammalian host can include attachment or adhesion to the host cell surface, invasion of host cells, and elaboration of toxins, for example. The involvement of pathogenic mechanisms or virulence factors of the microorganisms can result in deleterious or beneficial effect to the mammalian host or an asymptomatic or benign infection. In certain instances, this interaction can be nonspecific. In others, such microbial interaction involves the specific binding of the microorganism to a particular receptor or receptor complex expressed on the host cell surface. In turn, the binding event can trigger changes in the microorganism and/or the mammalian host cell, leading to the progression of infection.

Detailed Description Text (10):
In accordance with the present invention, a mammalian host preferably includes immunocompromised or immunosuppressed humans, for example, those having AIDS or undergoing transplantation or anti-cancer therapy. The invention also preferably relates to humans with primary or secondary immunodeficiencies (Merck Manual 16th ed., Chapter 19 (1992), herein incorporated by reference). In addition to mammalian hosts in which the normal immune response has been compromised or suppressed, the invention relates to mammalian hosts in which the normal microbial flora has been disrupted, for example, because of disease (e.g., hereditary, metabolic, infiltrative, or hematologic), trauma (e.g., bum, splenectomy, anesthesia), surgical or clinical procedure (e.g., catheterization or introduction of artificial implants such as dentures), or chemical, radiation, or other immunosuppressive prophylaxis or treatment. Accordingly, the microbial infection of the present invention includes infections related to opportunistic as well as pathogenic microorganisms.

Detailed Description Text (12):
The three-letter symbols used to represent the amino acid residues in the peptides of the present invention are those symbols commonly used in the art. The amino acid residues are preferred to be in the L isomeric form. However, residues in the D isomeric form may be substituted for any L-amino acid, as long as the desired functional property of inhibition of transglutaminase-mediated microbial interaction with a mammalian host is retained by the peptide. The three-letter symbols used herein refer to the following amino acids: Ser is serine; Ile is isoleucine; Gln is glutamine; Phe is phenylalanine; His is histidine; Trp is tryptophan; Lys is lysine; Asn is asparagine; Leu is leucine; Gly is glycine; Thr is threonine; Asp is aspartic acid; Arg is arginine; and Ala is alanine.

Detailed Description Text (13):
Polypeptides of the present invention may include any analog, fragment or chemical derivative of the polypeptides capable of inhibiting transglutaminase-mediated microbial interaction with a mammalian host. Polypeptides thus may include soluble peptides, Ig-tailed fusion peptides, members of random peptide libraries (see, e.g., Lam, K. S. et al., Nature 354:82-84 (1991); Houghten, R. et al., Nature 354:84-86 (1991)), combinatorial chemistry-derived molecular library made of D- and/or L-configuration amino acids, and phosphopeptides (including members of random or partially degenerate, directed phosphopeptide libraries, see, e.g., Songyang, Z. et al., Cell 72:767-778(1993)).

Detailed Description Text (24):
Washed M199-germinated C. albicans cells (10.sup.8 /ml), guinea pig liver transglutaminase (8.5 .mu.g), and 5-(biotinamido)pentylamine (30 .mu.M) (Pierce) were incubated in reaction buffer 1 (400 .mu.l) (100 mM Tris-Cl pH 7.5, 5 mM CaCl.sub.2, 1 mM DTT, 2 mM EDTA) for 15 minutes at 37.degree. C. Reactions were stopped with EDTA (10 mM), germ tubes were washed with distilled water, spotted onto microscope slides, air dried, incubated with avidin-FITC (1:100) (Zymed) and BSA-rhodamine (1:30) (Difco) in PBS at 37.degree. C., washed and examined by fluorescence microscopy.

Detailed Description Text (29):
A 365 base pair BglII-BclI fragment was deleted from pGBHWP1, a recombinant plasmid containing genomic HWP1 DNA in pBluescript SK- (Stratagene), and replaced with the hisG-URA3-hisG cassette from p5921 (W. A. Fonzi, M. Y. Irwin, Genetics 134:717-728 (1993)) to create pHWP1URA3. A Ura.sup.+ heterozygous hwp1/HWP1 strain, CAH7, was created by transformation of the ura3 auxotrophic strain CAI4 (Fonzi, supra) by spheroplast transformation (M. B. Kurtz, M. W. Cortelyou, D. R. Kirsch, Mol. Cell. Biol. 6:142-149 (1986)) with HindIII-digested pHWP1URA3. A Ura.sup.+ homozygous hwp1/hwp1 strain, CAH7-1A was created by transformation of a Ura.sup.- heterozygous hwp1/HWP1 strain, CAH7-1, that was derived from CAH7 following selection on 5-fluoroorotic acid (5-FOA)-containing medium (J. D. Boeke, F. Lacroute, G. R. Fink, Genet. 197:345-346 (1984)). An HWP1 revertant strain, CAHR3, was created by co-transformation of a Ura.sup.- homozygous hwp1/hwp1 strain, CAH7-1A1, with Hind III-digested pGBHWP1 and p24enura (P. Postlethwait, P. Sundstrom, J. Bacteriol. 177:1772-1779 (1995)) digested with Xba I-Xho I resulting in disruption of an enolase gene with URA3. Gene replacements at the HWP1 and enolase loci were confirmed by Southern blotting. Although CAHR3 contained excess HWP1 DNA, HWP1 MRNA levels were equivalent to those of CAH7 which contained a single HWP1 gene. The presence or absence of HWP1 in each strain was correlated with surface Hwp1 by indirect immunofluorescence assays.

Detailed Description Text (30):
HWP1 was disrupted by replacing 365 bp in the N-terminal coding region with a hisGURA3 cassette (FIGS. 3A, 3B). Homozygous hwp1/hwp1 strains lacked HWP1 mRNA (FIG. 3C) and Hwp1 on germ tube surfaces. An HWP1 revertant was prepared by complementation of a homozygous hwp1/hwp1 mutant strain with HWP1 creating CAHR3 (FIGS. 3A, 3B). Levels of CAHR3 mRNA were similar to those of the heterozygous hwp1/HWP1 strain CAH7 (FIG. 3C), whereas germ tube Hwp1 expression was indistinguishable from that of the other strains. Transglutaminase-mediated incorporation of a lysine analogue in the homozygous hwp1/hwp1 mutant strain CAH7-1A (FIG. 2E) was nearly absent as shown by the marked decrease in fluorescence compared to SC5314, CAI4, and CAH7 (FIGS. 2A, 2C, 2D). The existence of an additional, less

effective transglutaminase substrate was suggested by the weak fluorescence of CAH7-1A. Transglutaminase substrate activity on germ tubes was regained upon complementation of a homozygous hwp1/hwp1 mutant strain with HWP1 (FIG. 2F). Endogenous transglutaminase activity of C. albicans was not detected in this assay or in broken cell walls. The marked decrease in fluorescence of the homozygous hwp1/hwp1 mutant strain showed that Hwp1 is the major substrate for transglutaminase on germ tube surfaces.

Detailed Description Text (40):
(Hwp1 in Mammalian Transglutaminase-mediated Binding to Buccal Epithelial Cells)

Detailed Description Text (41):
To determine if Hwp1 cross-links C. albicans germ tubes to buccal epithelial cells during incubation at 37.degree. C., germtube-BEC complexes were heated to 100.degree. C. in the presence of SDS, causing dissociation of noncovalent bonds, but leaving germ tubes and BEC envelopes intact (FIG. 5C, top panel).

Detailed Description Text (42):
Mass conversion of yeasts, radiolabeled with Tran.sup.35 S-label (ICN) (5 .mu.Ci/ml), to germ tubes (5.times.10.sup.6 /ml) was induced in prewarmed M199 (40 ml) for 2.5 hours at 37.degree. C. No differences in the proportion of (>95%) germ tubes or in germ tube length were seen among strains. Washed germ tubes and BECs from a healthy donor, suspended in 300 .mu.l of reaction buffer 3 (50 mM Tris-Cl pH 7.5, 10 mM CaCl.sub.2, 1 mM EDTA, 1 mM DTT), at a germ tube/BEC ratio of 100:1, were mixed, incubated for 1 hour at 37.degree. C. followed by reaction termination with 100 mM EGTA (75 .mu.l). The BEC donors understood the nature of the studies and consented to provide BECs. Radioactivity of BEC fractions was determined by scintillation counting after centrifugation on 50% Percoll gradients, and germ tubes per BEC was determined using the specific activity of each strain. Background counts, determined from gradients with germ tubes only, were less than 6% of counts for germ tube/BEC mixtures and were subtracted from counts of epithelial cell-germ tube mixtures. For stabilized adhesion assays, reaction mixtures were heated to 100.degree. C. for 2 minutes in PBS containing 1% SDS prior to loading on Percoll gradients. To inhibit transglutaminase, BECs were pretreated in PBS containing iodoacetamide (10 mM) for 15 minutes at 37.degree. C., and suspended in reaction buffer supplemented with iodoacetamide (10 mM) prior to incubation with germ tubes.

Detailed Description Text (43):
UnoPP-1, a CAI4 derivative made Ura.sup.+ by disruption of an enolase gene with URA3 and having unaltered HWP1 genes (FIGS. 3B, 3C), served as a positive control for HWP1 mutant strains that were also derived from CAI4 and contained URA3. Adherence of UnoPP-1 was set at 100%. Stabilized adhesion of the heterozygous hwp1/HWP1 strain CAH7 and the HWP1 revertant strain CAHR3 were indistinguishable from each other and from UnoPP-1 (FIG. 5A). However, stabilized adhesion of the hwp1/hwp1 mutant strain CAH7-1A was only 23% of UnoPP-1. The low level of stabilized adhesion of CAH7-1A was equivalent to that of UnoPP-1 when iodoacetamide was added to inhibit transglutaminase. These results show that C. albicans becomes cross-linked to epithelial cell envelopes and that Hwp1 is responsible for cross-linking.

Detailed Description Text (45):
(Inhibition of the Adhesion of C. albicans Germ Tubes to Human Buccal Epithelial Cells with Monodansylcadaverine)

Detailed Description Text (47):
Radiolabeled germ tubes of the control strain, UnoPP-1, were incubated with BECs at a ratio of 100:1 with or without 5 or 10 mM monodansylcadaverine in reaction buffer 3, supra. The monodansylcadaverine was added last to the reaction mix from a 100 mM stock solution prepared in DMSO and kept at room temperature, protected from light. An equal volume of DMSO was included in positive and background control tubes to assess any effects of DMSO on the adhesion assay. The cell mixtures were incubated for 1 hour at 37.degree. C., and the number of germ tubes covalently attached to BECs were determined. The effect of monodansylcadaverine on the adhesion of germ tubes to BEC was determined relative to the control samples with DMSO alone. The inclusion of 5 or 10 mM monodansylcadaverine reduced the adhesion by approximately 87%, whereas DMSO alone had no effect on the adhesion of germ tubes to BEC. The

ability to inhibit stabilized adhesion by monodansylcadaverine indicates the therapeutic usefulness of transglutaminase substrates in inhibition of adhesion of C. albicans to BECs.

Detailed Description Text (49):
(Role of Hwp1 in the Adhesion of C. albicans Germ Tubes to Human Buccal Epithelial Cells)

Detailed Description Text (54):
The importance of Hwp1 in candidiasis is supported by experiments showing that the hwp1 mutant strain CAH7-1A has a greatly reduced capacity to cause systemic candidiasis in mice compared to strains expressing HWP1. Upon intravenous inoculation, five of six mice injected with the homozygous hwp1/hwp1 mutant strain CAH7-1A were alive at 30 days compared to only two of eighteen mice given HWP1-expressing strains (FIG. 6). Interestingly, the survival curve of the revertant closely approximated that of the wild type control SC5314, providing a clear role for Hwp1 in pathogenesis. Invasion associated with systemic candidiasis may be exacerbated through interactions of surface Hwp1 with a plasma transglutaminase, factor XIII or clot stabilizing factor. These results strongly implicate a central role for Hwp1 in the pathogenesis of transglutaminase-mediated infections such as candidiasis.

Detailed Description Text (59):
Of course, the present polypeptides may also be prepared by recombinant DNA techniques. The present invention also relates to vectors comprising DNA molecules of the present invention, host cells which are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques. Host cells may be genetically engineered (transduced or transformed or transfected) with the vectors of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying genes. The culture conditions, such as temperature, pH and the like, are preferably those previously used with the host cell selected for expression, and will be apparent to the skilled artisan.

Detailed Description Text (64):
The DNA sequence in the expression vector may be operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be mentioned: LTR or SV40 promoter, the E. coli. lac or trp, the phage lambda P.sub.L promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression.

Detailed Description Text (65):
In addition, the expression vectors preferably may contain a gene to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in E. coli.

Detailed Description Text (66):
An embodiment of the invention is an isolated DNA molecule comprising the nucleotide sequence of SEQ. ID NO. 2 (FIG. 7). This nucleotide sequence, or fragments or functional equivalents thereof, may be used to generate recombinant DNA molecules that direct the expression of the polypeptides of the present invention, or functionally active peptides or functional equivalents thereof, in appropriate host cells. Due to the degeneracy of the nucleotide coding sequence, other DNA sequences which encode substantially the same amino acid sequences as depicted in SEQ. ID NO. 1, or analogs or fragments thereof, may be used in the practice of the invention for the cloning and expression of a substrate for mammalian transglutaminases. Such alterations include deletions, additions or substitutions of different nucleotide residues resulting in a sequence that encodes the same or a functionally equivalent gene product. The gene product may contain deletions, additions or substitutions of

amino acid residues within the sequence, which result in a silent change thus producing a bioactive product. Such amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, the amphipathic nature of the residues involved and/or on the basis of crystallographic data. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; amino acids with uncharged polar head groups having similar hydrophilicity values include the following: leucine, isoleucine, valine; glycine, alanine; asparagine, glutamine; serine, threonine; phenylalanine, tyrosine.

Detailed Description Text (70):
The vector containing the appropriate DNA sequence, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the polypeptides of the present invention. Representative examples of appropriate hosts include: bacterial cells, such as E. coli, Salmonella typhimurium, Streptomyces; fungal cells, such as yeast; insect cells, such as Drosophila S2 and Spodoptera Sf9; animal cells, such as CHO, COS or Bowes melanoma; adenoviruses; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art.

Detailed Description Text (74):
(Cells Transformed with Recombinant Vectors Containing DNA Encoding Polypeptide Substrates for Mammalian Transglutaminases)

Detailed Description Text (75):
In a further embodiment, the present invention relates to host cells containing the above-described construct. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. The host cell preferably may secrete the recombinant protein. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (L. Davis et al., Basic Methods in Molecular Biology, 1986)).

Detailed Description Text (76):
The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers. Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., supra.

Detailed Description Text (78):
Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), alpha factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is preferably assembled in appropriate phase with translation, initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Detailed Description Text (81):
Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter may be de-repressed by appropriate means (e.g., temperature shift or chemical induction) and cells may be cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained

for further purification.

Detailed Description Text (82):
Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents. Various mammalian cell culture systems can also be employed to express recombinant polypeptides. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell, 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors may comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation sites, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early promoter, enhancer, splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

Detailed Description Text (83):
The polypeptides of the present invention may be recovered and purified from recombinant cell cultures by methods used heretofore, including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxyapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

Detailed Description Text (84):
The polypeptides of the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated with mammalian or other eukaryotic carbohydrates or may be non-glycosylated. Polypeptides of the invention may also include an initial methionine amino acid residue.

Detailed Description Text (91):
For use in a method of identification, prevention or treatment, such as the identification, prevention or treatment of infection of a mammalian host by a microorganism, the polypeptides of the present invention may be present in a pharmaceutical composition in admixture with a pharmaceutically acceptable sterile vehicle. The pharmaceutical composition may be compounded according to conventional pharmaceutical formulation techniques.

Detailed Description Text (98):
Another embodiment of the present invention relates to a monoclonal antibody to the polypeptides of the present invention (or an antigenic portion thereof), which may be produced by methods recognized in the art, including the formation of monoclonal antibody-producing hybridomas (Kohler, G., and C. Milstein, Nature 256:495-497 (1975); Eur. J. Immunol. 6:511-519 (1976)). By fusing antibody-forming cells (spleen lymphocytes) with myeloma cells (malignant cells of bone marrow primary tumors), a hybrid cell line is created from a single fused cell hybrid (called a hybridoma or clone) having certain inherited characteristics of both the lymphocytes and myeloma cell lines. Like the lymphocytes (taken from animals primed with sheep red blood cells as antigen), the hybridomas secreted a single type of immunoglobulin specific to the antigen; moreover, like the myeloma cells, the hybrid cells had the potential for indefinite cell division. The combination of these two features offered distinct advantages over conventional antisera. Whereas antisera derived from vaccinated animals are variable mixtures of polyclonal antibodies which never can be reproduced identically, monoclonal antibodies are highly specific immunoglobulins of a single type. The single type of immunoglobulin secreted by a hybridoma is specific to one and only one antigenic determinant, or epitope, on the antigen, a complex molecule having a multiplicity of antigenic determinants. For instance, if the antigen is a protein, an antigenic determinant may be one of the many peptide sequences

(generally 6-7 amino acids in length (Atassi, M. Z., Molec. Cell. Biochem. 32:21-43 (1980)) within the entire protein molecule. Hence, monoclonal antibodies raised against a single antigen may be distinct from each other depending on the determinant that induced their formation; but for any given clone, all of the antibodies it produces are identical. Furthermore, the hybridoma cell line can be reproduced indefinitely, is easily propagated in vitro or in vivo, and yields monoclonal antibodies in extremely high concentration.

Detailed Description Text (102):
In an embodiment of the invention, purified polypeptides of the present invention (or an antigenic portion thereof) can be used as an antigen or immunogen. In addition, microorganisms expressing Hwp1 protein or polypeptide fragments thereof also represent potential antigens or sources of antigen with which to immunize animals to obtain somatic cells for fusion. Somatic cells with the potential for producing antibody and, in particular, B lymphocytes, are suitable for fusion with a B-cell myeloma line. Those antibody-producing cells that are in the dividing plasmablast stage fuse preferentially. Somatic cells may be derived from the lymph nodes, spleens and peripheral blood of primed animals and the lymphatic cells of choice depending to a large extent on their empirical usefulness in the particular fusion system. Once-primed or hyperimmunized animals can be used as a source of antibody-producing lymphocytes. Mouse lymphocytes give a higher percentage of stable fusions with mouse myeloma lines. However, the use of rat, rabbit, and frog cells is also possible. Alternatively, human somatic cells capable of producing antibody, specifically B lymphocytes, are suitable for fusion with myeloma cell lines. While B lymphocytes from biopsied spleens or lymph nodes of individual may be used, the more easily accessible peripheral blood B lymphocytes are preferred. The lymphocytes may be derived from patients with diagnosed carcinomas.

Detailed Description Text (103):
Specialized myeloma cell lines have been developed from lymphocyte tumors for use in hybridoma-producing fusion procedures (Kohler, G., and C. Milstein, Eur. J. Immunol. 6:511-519 (1976); M. Schulman et al., Nature 276: 269-270 (1978)). Examples of myeloma cell lines that may be used for the production of fused cell hybrids include X63-Ag8, NSI-Ag4/1, MPC11-45.6TG1.7, C63-Ag8.653, Sp2/0-Ag14, FO, and S194/5XX0.BU.1, all derived from mice; 210.RCY3.Ag1.2.3, U-226AR, and GM1500GTGAL2, all derived from rats; and U-226AR and GM1500GTGAL2, derived from humans, (G. J. Hammerling, U. Hammerling, and J. F. Kearney (editors), Monoclonal Antibodies and T-cell Hybridomas in: J. L. Turk (editor) Research Monographs in Immunology, Vol. 3, Elsevier/North Holland Biomedical Press, NY (1981)).

Detailed Description Text (104):
Methods for generating hybrids of antibody-producing spleen or lymph node cells and myeloma cells usually comprise mixing somatic cells with myeloma cells in a 2:1 proportion (though the proportion may vary from about 20:1 to about 1:1), respectively, in the presence of an agent or agents (chemical or electrical) that promote the fusion of cell membranes. It is often preferred that the same species of animal serve as the source of the somatic and myeloma cells used in the fusion procedure. Fusion methods have been described by Kohler and Milstein (Nature 256:495-497 (1975) and Eur. J. Immunol. 6:511-519 (1976), and by Gefter et al. (Somatic Cell Genet. 3:231-236 (1977)). The fusion-promotion agents used by those investigators were Sendai virus and polyethylene glycol (PEG), respectively.

Detailed Description Text (105):
Generally, the fused cells are cultured in selective media, for instance HAT medium containing hypoxanthine, aminopterin and thymidine. HAT medium permits the proliferation of hybrid cells and prevents growth of unfused myeloma cells which normally would continue to divide indefinitely. Aminopterin blocks de novo purine and pyrimidine synthesis by inhibiting the production of tetrahydrofolate. The addition of thymidine bypasses the block in pyrimidine synthesis, while hypoxanthine is included in the media so that inhibited cells synthesize purine using the nucleotide salvage pathway. The myeloma cells employed are mutants lacking hypoxanthine phosphoribosyl transferase (HPRT) and thus cannot utilize the salvage pathway. In the surviving hybrid, the B lymphocyte supplies genetic information for production of this enzyme. Since B lymphocytes themselves have a limited life span in culture (approximately two weeks), the only cells which can proliferate in HAT

media are hybrids formed from myeloma and spleen cells.

Detailed Description Text (107):
Once the desired fused cell hybrids have been selected and cloned into individual antibody-producing cell lines, each cell line may be propagated in either of two standard ways. A sample of the hybridoma can be injected into a histocompatible animal of the type that was used to provide the somatic and myeloma cells for the original fusion. The injected animal develops tumors secreting the specific monoclonal antibody produced by the fused cell hybrid. The body fluids of the animal, such as serum or ascites fluid, can be tapped to provide monoclonal antibodies in high concentration. Alternatively, the individual cell lines may be propagated in vitro in laboratory culture vessels; the culture medium, also containing high concentrations of a single specific monoclonal antibody, can be harvested by decantation, filtration or centrifugation.

Detailed Description Text (112):
The use of the monoclonal antibodies described herein can be extended to the screening of human biological fluids for the presence of the specific antigenic determinant recognized. In vitro immunoserological evaluation of sera withdrawn from patients thereby permits non-invasive diagnosis of microbial infection. By way of illustration, human fluids, such as pleural fluids or lymph, can be taken from a patient and assayed for the specific epitope, either as released antigen or membrane-bound on cells in the sample fluid, using monoclonal antibodies against the polypeptides of the present invention in standard radioimmunoassays or enzyme-linked immunoassays known in the art or competitive binding enzyme-linked immunoassays.

Detailed Description Text (113):
The monoclonal antibodies of this invention are potentially useful for targeting microbial infection in vivo. They can therefore be used in humans for localization and monitoring of the microbial infection. For this application, it is preferable to use purified monoclonal antibodies. Purification of monoclonal antibodies for human administration by ammonium sulfate or sodium sulfate precipitation followed by dialysis against saline and filtration sterilization has been described by Miller et al. (in: Hybridomas in Cancer Diagnosis and Therapy, (1982), p. 134).

Detailed Description Text (114):
Alternatively, immunoaffinity chromatography techniques may be used to purify the monoclonal antibodies. The purified monoclonal antibodies can be labeled with radioactive compounds, for instance, radioactive iodine, and administered to a patient intravenously. After localization of the antibodies at the infection site, they can be detected by emission tomographical and radionuclear scanning techniques, thereby pinpointing the location of the infection. Experimental radioimmunodetection with monoclonal antibodies may occur by external scintigraphy.

Detailed Description Text (127):
Another embodiment of the present invention involves the use of the DNA of the present invention in gene therapy applications. Gene therapy has been broadly defined as "the correction of a disease phenotype through the introduction of new genetic information into the affected organism" (Roemer, K. and Friedmann, T., Eur. J. Biochem. 208: 211-225 (1992)). Two basic approaches to gene therapy have evolved: (1) ex vivo gene therapy and (2) in vivo gene therapy. In ex vivo gene therapy, cells are removed from a subject and cultured in vitro. A functional replacement gene is introduced into the cells (transfection) in vitro, the modified cells are expanded in culture, and then reimplanted in the subject. These genetically modified, reimplanted cells are reported to secrete detectable levels of the transfected gene product in situ (Miller, A. D., Blood 76: 271-278 (1990)) and Selden, R. F., et al., New Eng. J. Med. 317: 1067-1076 (1987)). The development of improved retroviral gene transfer methods (transduction) facilitates the transfer into and subsequent expression of genetic material by somatic cells (Cepko, C. L., et al., Cell 37: 1053-1062 (1984)). Accordingly, retrovirus-mediated gene transfer has been used in clinical trials to mark autologous cells and as a way of treating genetic disease (Rosenberg, S. A., et al., New Eng. J. Med. 323: 570-578 (1990); Anderson, W. F., Human Gene Therapy 2: 99-100 (1991)). Several ex vivo gene therapy studies in humans are reported (reviewed in Anderson, W. F., Science 256: 808-813 (1992) and Miller A. D., Nature 357: 455-460 (1992)).

Detailed Description Text (128):
In in vivo gene therapy, target cells are not removed from the subject. Rather, the transferred gene is introduced into cells of the recipient organism in situ, that is, within the recipient. In vivo gene therapy has been examined in several animal models (reviewed in Felgner, P. L. and Rhodes, G., Nature 349: 351-352 (1991)). Publications have reported the feasibility of direct gene transfer in situ into organs and tissues such as muscle (Ferry, N., et al., Proc. Natl. Acad. Sci. 88: 8377-8781 (1991); Quantin, G., et al., Proc. Natl. Acad. Sci. USA 89: 2581-2584 (1992)), hematopoietic stem cells (Clapp, D. W., et al., Blood 78: 1132-1139 (1991)), the arterial wall (Nabel, E. G., et al., Science 244: 1342-1344 (1989)), the nervous system (Price, J. D., et al., Proc. Natl. Acad. Sci. 84: 156-160 (1987)), and lung (Rosenfeld, M. A., et al., Science 252: 431-434 (1991)). Direct injection of DNA into skeletal muscle (Wolff, J. A., et al., Science 247: 1465-1468 (1990)), heart muscle (Kitsis, R. N., et al., Proc. Natl. Acad. Sci. USA 88: 4138-4142 (1991)) and injection of DNA-lipid complexes into the vasculature (Lim, C. S., et al., Circulation 83: 2007-2011 (1991); Ledere, G. D., et al., J. Clin. Invest. 90: 936-944 (1992); Chapman, G. D., et al., Circ. Res. 71: 27-33 (1992)) also have been reported to yield a detectable expression level of the inserted gene product(s) in vivo.

Detailed Description Text (129):
Recent gene therapy efforts have been aimed at the identification of various cell types for transformation, including keratinocytes (Morgan, J. R., et al., Science 237: 1476-1479 (1987)), fibroblasts (Palmer, T. D., et al., Proc. Natl. Acad. Sci. 88: 1330-1334 (1991); Garver Jr., R. I., et al., Science 237: 762-764 (1987); International Patent Application PCT/US92/01890, having publication number WO92/15676), lymphocytes (Reimann, J. K., et al., J. Immunol. Methods 89: 93-101 (1986)), myoblasts (Barr, E. and Leiden, J. M., Science 254: 1507-1509 (1991); Dai, Y. et al., PNAS 89: 10892-10895 (1992); Roman, M., et al., Somatic Cell and Molecular Genetics 18: 247-258 (1992)), smooth muscle cells (Lynch, C. M. et al., Proc. Natl. Acad. Sci. USA 89: 1138-1142 (1992)), and epithelial cells (Nabel, E. G., et al., Science 244: 1342-1344 (1989)), International Patent Application PCT/US89/05575 (having publication number WO 90/06997), the contents of which references and patent/patent applications are incorporated herein by reference.

Detailed Description Text (130):
The delivery of an effective dose of a prophylactic or therapeutic agent in situ depends on the efficiency of transfection (or transduction) as well as the number of target cells. Epithelial cell-based gene therapy, in particular, involves a relatively small area available in situ for receiving genetically modified epithelial cells. The delivery of an effective dose of prophylactic or therapeutic agent in situ thus depends upon the total number of implanted epithelial cells.

Detailed Description Text (131):
In one embodiment of the invention, exogenous genetic material (e.g., a cDNA encoding a polypeptide of the present invention) is introduced into a syngeneic host cell ex vivo or in vivo by genetic transfer methods, such as transfection or transduction, to provide a genetically modified host cell. Various expression vectors (i.e., vehicles for facilitating delivery of exogenous genetic material into a target cell) are known to one skilled in the art.

Detailed Description Text (132):
Transfection refers to the insertion of nucleic acid into a mammalian host cell using physical or chemical methods. Several transfection techniques are known to those of ordinary skill in the art including: calcium phosphate DNA co-precipitation (Methods in Molecular Biology, Vol. 7, Gene Transfer and Expression Protocols, Ed. E. J. Murray, Humana Press (1991)); DEAE-dextran; electroporation; cationic liposome-mediated transfection; and tungsten particle-facilitated microparticle bombardment (Johnston, S. A., Nature 346: 776-777 (1990)). Strontium phosphate DNA co-precipitation (Brash D. E. et al. Molec. Cell. Biol. 7: 2031-2034 (1987)) is a preferred transfection method.

Detailed Description Text (133):
In contrast, transduction refers to the process of transferring nucleic acid into a

cell using a DNA or RNA virus. A RNA virus (i.e., a retrovirus) for transferring a nucleic acid into a cell is referred to herein as a transducing chimeric retrovirus. Exogenous genetic material contained within the retrovirus is incorporated into the genome of the transduced host cell. A host cell that has been transduced with a chimeric DNA virus (e.g., an adenovirus carrying a cDNA encoding a therapeutic agent) will not have the exogenous genetic material incorporated into its genome, but will be capable of expressing the exogenous genetic material that is retained extrachromosomally within the cell.

Detailed Description Text (134):
Typically, the exogenous genetic material includes the heterologous gene (usually in the form of a cDNA comprising the exons coding for the therapeutic protein) together with a promoter to control transcription of the new gene. The promoter characteristically has a specific nucleotide sequence necessary to initiate transcription. Optionally, the exogenous genetic material further includes additional sequences (i.e., enhancers) required to obtain the desired gene transcription activity. For the purpose of this discussion an enhancer is simply any non-translated DNA sequence which works contiguous with the coding sequence (in cis) to change the basal transcription level dictated by the promoter. Preferably, the exogenous genetic material is introduced into the host cell genome immediately downstream from the promoter so that the promoter and coding sequence are operatively linked so as to permit transcription of the coding sequence. A preferred retroviral expression vector includes an exogenous promoter element to control transcription of the inserted exogenous gene. Such exogenous promoters include both constitutive and inducible promoters.

Detailed Description Text (135):
Naturally-occurring constitutive promoters control the expression of essential cell functions. As a result, a gene under the control of a constitutive promoter is expressed under all conditions of cell growth. Exemplary constitutive promoters include the promoters for the following genes which encode certain constitutive or housekeeping functions: hypoxanthine phosphoribosyl transferase (HPRT), dihydrofolate reductase (DHFR) (Scharfmann et al., Proc. Natl. Acad. Sci. USA 88: 4626-4630 (1991)), adenosine deaminase, phosphoglycerol kinase (PGK), pyruvate kinase, phosphoglycerol mutase, the beta -actin promoter (Lai et al., Proc. Natl. Acad. Sci. USA 86: 10006-10010 (1989)), and other constitutive promoters known to those of skill in the art. In addition, many viral promoters function constitutively in eukaryotic cells. These include: the early and late promoters of SV40, the long terminal repeats (LTRS) of Moloney Leukemia Virus and other retroviruses, and the thymidine kinase promoter of Herpes Simplex Virus, among many others. Accordingly, any such constitutive promoters can be used to control transcription of a heterologous gene insert.

Detailed Description Text (136):
Genes that are under the control of inducible promoters are expressed only or to a greater degree, in the presence of an inducing agent, (e.g., transcription under control of the metallothionein promoter is greatly increased in presence of certain metal ions). Inducible promoters include responsive elements (REs) which stimulate transcription when their inducing factors are bound. For example, there are REs for serum factors, steroid hormones, retinoic acid and cyclic AMP. Promoters containing a particular RE can be chosen in order to obtain an inducible response, and in some cases, the RE itself may be attached to a different promoter, thereby conferring inducibility to the recombinant gene. Thus, by selecting the appropriate promoter (constitutive versus inducible; strong versus weak), it is possible to control both the existence and level of expression of a therapeutic agent in the genetically modified host cell. If the gene encoding the prophylactic or therapeutic agent is under the control of an inducible promoter, delivery of the agent in situ is triggered by exposing the genetically modified cell in situ to conditions for permitting transcription of the prophylactic or therapeutic agent, e.g., by intraperitoneal injection of specific inducers of the inducible promoters which control transcription of the agent. For example, in situ expression by genetically modified host cells of a therapeutic agent encoded by a gene under the control of the metallothionein promoter, is enhanced by contacting the genetically modified cells with a solution containing the appropriate (i.e., inducing) metal ions in situ.

Detailed Description Text (137):
Accordingly, the amount of therapeutic agent that is delivered in situ is regulated by controlling such factors as: (1) the nature of the promoter used to direct transcription of the inserted gene (i.e., whether the promoter is constitutive or inducible, strong or weak); (2) the number of copies of the exogenous gene that are inserted into the host cell; (3) the number of transduced/transfected host cells that are administered (e.g., implanted) to the patient; (4) the size of the implant (e.g., graft or encapsulated expression system); (5) the number of implants; (6) the length of time the transduced/transfected cells or implants are left in place; and (7) the production rate of the prophylactic or therapeutic agent by the genetically modified host cell. Selection and optimization of these factors for delivery of an effective dose of a particular prophylactic or therapeutic agent is deemed to be within the scope of one of skill in the art, taking into account the above-disclosed factors and the clinical profile of the patient.

Detailed Description Text (138):
In addition to at least one promoter and at least one heterologous nucleic acid encoding the prophylactic or therapeutic agent, the expression vector preferably includes a selection gene, for example, a neomycin resistance gene, for facilitating selection of host cells that have been transfected or transduced with the expression vector. Alternatively, the host cells are transfected with two or more expression vectors, at least one vector containing the gene(s) encoding the prophylactic or therapeutic agent(s), the other vector containing a selection gene. The selection of a suitable promoter, enhancer, selection gene and/or signal sequence is deemed to be within the scope of one skilled in the art.

Detailed Description Text (139):
The prophylactic or therapeutic agent can be targeted for delivery to an extracellular, intracellular or membrane location. If it is desirable for the gene product to be secreted from the host cells, the expression vector is designed to include an appropriate secretion signal sequence for secreting the therapeutic gene product from the cell to the extracellular milieu. If it is desirable for the gene product to be retained within the host cell, this secretion signal sequence is omitted. In a similar manner, the expression vector can be constructed to include retention signal sequences for anchoring the prophylactic or therapeutic agent within the host cell plasma membrane. For example, membrane proteins have hydrophobic transmembrane regions that stop translocation of the protein in the membrane and do not allow the protein to be secreted. The construction of an expression vector including signal sequences for targeting a gene product to a particular location is deemed to be within the scope of one of skill in the art.

Detailed Description Text (140):
In an embodiment, vectors for mammalian host cell gene therapy are viruses, more preferably replication-deficient viruses (described in detail below). Exemplary viral vectors are derived from: Harvey Sarcoma virus; ROUS Sarcoma virus, MPSV, Moloney murine leukemia virus and DNA viruses (e.g., adenovirus) (Temin, H., Retrovirus vectors for gene transfer, in Gene Transfer, Kucherlapati R, Ed., pp. 149-187, Plenum, (1986)).

Detailed Description Text (141):
Replication-deficient retroviruses are capable of directing synthesis of virion proteins, but are incapable of making infectious particles. Accordingly, these genetically altered retroviral expression vectors have general utility for high-efficiency transduction of genes in cultured cells, and specific utility for use in the method of the present invention. Such retroviruses further have utility for the efficient transduction of genes into host cells in vivo. Retroviruses have been used extensively for transferring genetic material into cells. Standard protocols for producing replication-deficient retroviruses (including the steps of incorporation of exogenous genetic material into a plasmid, transfection of a packaging cell line with plasmid, production of recombinant retroviruses by the packaging cell line, collection of viral particles from tissue culture media, and infection of the target cells with the viral particles) are provided in Kriegler, M. Gene Transfer and Expression, A Laboratory Manual, W. H. Freeman Co., NY (1990) and Murray, E. J., ed. Methods in Molecular Biology, Vol. 7, Humana Press Inc., Clifton,

N.J. (1991).

Detailed Description Text (142):
The major advantage of using retroviruses for gene therapy is that the viruses insert the gene encoding the therapeutic agent into the host cell genome, thereby permitting the exogenous genetic material to be passed on to the progeny of the cell when it divides. In addition, gene promoter sequences in the LTR region have been reported to enhance expression of an inserted coding sequence in a variety of cell types (see e.g., Hilberg et al., Proc. Natl. Acad. Sci. USA 84: 5232-5236 (1987); Holland et al., Proc. Natl. Acad. Sci. USA 84: 8662-8666 (1987); Valerio et al., Gene 84: 419-427 (1989)). In vivo gene therapy using replication-deficient retroviral vectors to deliver a therapeutically effective amount of a therapeutic agent can be efficacious if the efficiency of transduction is high and/or the number of target cells available for transduction is high.

Detailed Description Text (143):
Yet another viral candidate useful as an expression vector for transformation of mammalian host cells is the adenovirus, a double-stranded DNA virus. The adenovirus is frequently responsible for respiratory tract infections in humans and thus appears to have an avidity for the epithelium of the respiratory tract (Straus, S., The Adenovirus, H. S. Ginsberg, Editor, Plenum Press, NY, p.451-496 (1984)). Moreover, the adenovirus is infective in a wide range of cell types, including, for example, muscle and epithelial cells (Larrick, J. W. and Burck, K. L., Gene Therapy. Application of Molecular Biology, Elsevier Science Publishing Co., Inc., NY, p.71-104 (1991)). The adenovirus also has been used as an expression vector in muscle cells in vivo (Quantin, B., et al., Proc. Natl. Acad. Sci. USA 89: 2581-2584 (1992)).

Detailed Description Text (145):
Thus, as will be apparent to one skilled in the art, a variety of suitable viral expression vectors are available for transferring exogenous genetic material into mammalian host cells. The selection of an appropriate expression vector to express an agent for the identification, prevention or treatment of microbial infection amenable to gene replacement therapy and the optimization of the conditions for insertion of the selected expression vector into the cell are within the scope of one of skill in the art without the need for undue experimentation.

Detailed Description Text (146):
In an alternative embodiment, the expression vector is in the form of a plasmid, which is transferred into the target host cells by one of a variety of methods: physical (e.g., microinjection (Capecchi, M. R., Cell 22: 479-488 (1980)), electroporation (Andreason, G. L. and Evans, G. A. Biotechniques 6: 650-660 (1988)), scrape loading, microparticle bombardment (Johnston, S. A., Nature 346: 776-777 (1990)) or by cellular uptake as a chemical complex (e.g., calcium or strontium coprecipitation, complexation with lipid, complexation with ligand) (Methods in Molecular Biology, Vol. 7, Gene Transfer and Expression Protocols, Ed. E. J. Murray, Humana Press (1991)). Several commercial products are available for cationic liposome complexation including Lipofectin (Life Technologies, Inc., Gaithersburg, Md.) (Felgner, P. L., et al., Proc. Natl. Acad. Sci. 84: 7413-7417 (1987)) and Transfectam.TM. (ProMega, Madison, Wis.) (Behr, J. P., et al., Proc. Natl. Acad. Sci. USA 86: 6982-6986 (1989); Loeffler, J. P., et al., J. Neurochem. 54: 1812-1815 (1990)). However, the efficiency of transfection by these methods is highly dependent on the nature of the target cell and accordingly, the conditions for optimal transfection of nucleic acids into host cells using the above-mentioned procedures must be optimized. Such optimization is within the scope of one of skill in the art.

Detailed Description Text (147):
In an embodiment, the preparation of genetically modified host cells contains an amount of cells sufficient to deliver a prophylactically or therapeutically effective dose of a substrate for mammalian transglutaminases of the present invention to the recipient in situ. The determination of an effective dose of the prophylactic or therapeutic agent for a known microbial infection is within the scope of one of skill in the art. Thus, in determining the effective dose, the skilled artisan would consider the condition of the patient, the severity of the

condition, as well as the results of clinical studies of the prophylactic or therapeutic agent being administered.

Detailed Description Text (148):
If the genetically modified host cells are not already present in a pharmaceutically acceptable carrier, they are placed in such a carrier prior to administration to the recipient. Such pharmaceutically acceptable carriers include, for example, isotonic saline and other buffers as appropriate to the patient and therapy. The genetically modified cells are administered by, for example, intraperitoneal injecting or implanting the cells or a graft or capsule containing the cells in a host cell-compatible site of the recipient. As used herein, host cell-compatible site refers to a structure, cavity or fluid of the recipient into which the genetically modified cell(s), host cell graft, or encapsulated host cell expression system can be implanted, without triggering adverse physiological consequences. Representative host cell-compatible sites include, for example, the peritoneal, pleural and pericardial cavities. Preferably, the host cell-compatible site communicates with the lymphatic system, thereby enabling delivery of the therapeutic agent to the vascular system.

Detailed Description Text (149):
In one embodiment, the host cell-compatible site may be denuded prior to implanting the cells. Exemplary denuding methods include but are not limited to: (1) injection of distilled water into the site (e.g., the peritoneal cavity) for 20 minutes, followed by scraping off a portion of the epithelial layer; (2) injection of 0.1% buffered trypsin for 20 minutes followed by scraping; (3) removal of epithelial cells by gentle scraping with a cell scraper and (4) touching a piece of Gelfilm (Upjohn, Kalamazoo, Mich.) to the endothelium.

Detailed Description Text (150):
The genetically modified host cells are implanted in a host cell-compatible site, alone or in combination with other genetically modified host cells. Thus, the instant invention embraces a method for modifying the epithelial system of a recipient by using a mixture of genetically modified host cells, such that a first modified cell expresses a first prophylactic or therapeutic agent of the present invention and a second modified cell expresses a second prophylactic or therapeutic agent. Other genetically modified cell types (e.g., hepatocytes, smooth muscle cells, fibroblasts, glial cells, mesothelial cells or keratinocytes) can be added, together with the genetically altered epithelial cells, to produce expression of a complex set of introduced genes. Moreover, more than one recombinant gene can be introduced into each genetically modified cell on the same or different vectors, thereby allowing the expression of multiple prophylactic or therapeutic agents of the present invention by a single cell.

Detailed Description Text (151):
The instant invention further embraces an epithelial cell graft. The graft comprises a plurality of the above-described genetically modified cells attached to a support that is suitable for implantation into a mammalian recipient, preferably into the oral cavity. The support can be formed of a natural or synthetic material. According to another aspect of the invention, an encapsulated host cell expression system is provided. The encapsulated system includes a capsule suitable for implantation into a mammalian recipient and a plurality of the above-described genetically modified host cells contained therein. The capsule can be formed of a synthetic or naturally-occurring material. The formulation of such capsules is known to one of ordinary skill in the art. In contrast to the host cells that are directly implanted into the mammalian recipient (i.e., implanted in a manner such that the genetically modified cells are in direct physical contact with the host cell-compatible site), the encapsulated cells remain isolated (i.e., not in direct physical contact with the site) following implantation. Thus, the encapsulated host cell system is not limited to a capsule including genetically-modified non-immortalized host cells, but may contain genetically modified immortalized host cells.

Other Reference Publication (11):
P. Sundstrom et al., Infection and Immunity, 55(3), 616-620 (Mar. 1987).

WEST Search History

DATE: Friday, November 07, 2003

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,PGPB,JPAB,EPAB,DWPI,TDBD; PLUR=YES; OP=OR			
L16	L15 and antimicrobial	412	L16
L15	L14 and (powder or ointment or paste or cream)	1692	L15
L14	L13 and infection	2585	L14
L13	L12 and eukaryotic adj4 cell	3439	L13
L12	L10 and (microbe$ or microbial) and cell	6186	L12
L11	L10 and (microbe$ or microbial) and $cell$	6139	L11
L10	isoleucine	26310	L10
L9	(microbe or microbial) adj5 isoleucine	13	L9
L8	microb$ same cell same isoleucine	89	L8
L7	L6 and (cell or eukaryot$ adj3 cell)	20	L7
L6	L5 and infection	29	L6
L5	microbial same isoleucine	124	L5
L4	L3 and (powder or paste or ointment or cream)	16	L4
L3	microbial and eukaryotic same isoleucine	117	L3
L2	L1 same isoleucine	3	L2
L1	microbial same eukaryotic	1339	L1

END OF SEARCH HISTORY

WEST Search History

DATE: Friday, November 07, 2003

Set Name side by side	Query	Hit Count	Set Name result set
	DB=USPT,PGPB,JPAB,EPAB,DWPI,TDBD; PLUR=YES; OP=OR		
L8	microb$ same cell same isoleucine	89	L8
L7	L6 and (cell or eukaryot$ adj3 cell)	20	L7
L6	L5 and infection	29	L6
L5	microbial same isoleucine	124	L5
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L3	microbial and eukaryotic same isoleucine	117	L3
L2	L1 same isoleucine	3	L2
L1	microbial same eukaryotic	1339	L1

END OF SEARCH HISTORY